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CULTURE MEDIA USED IN A DIAGNOSTIC MEDICAL MYCOLOGY LABORATORY

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While the bacteriologist depends upon biochemical tests primarily for the identification of many bacteria, the mycologist depends upon the gross and microscopic appearances of fungi for their identification. Because of deficiencies in certain growth factors, they frequently fail to develop all their characteristic features and, thus, are difficult to identify without the use of media including those growth factors for which there is a need.

Within the past few years much light has been thrown on the nutritional requirements of pathogenic fungi by such workers as Benham, Georg, Hazen, Langeron and Nickerson. If one reads the publications of these authors he will find formulae for media that will assist him in isolating and identifying these fungi. Many technologists, however, do not have access to these references; hence, it is sincerely hoped that this paper may assist the medical technologist studying fungi and encourage him to go on in his work rather than resigning himself to sending the culture to some one else for identification. The identification of fungi can be great fun and most rewarding if one will not become easily discouraged by terms, nomenclature, and technics. A medical technologist who has observed fungi under the microscope has seen some unequaled examples of beauty in the field of microbiology.

CLASSIFICATION: The fungi giving rise to infections in man are divided into four groups. The first of these include the superficial fungi which do not invade the skin, living only on its surface. It is not necessary to culture these organisms since the microscopic examination of skin scrapings are characteristic for

* 1st ASMT Award, Annual Convention, 1954, Miami Beach, Florida.

each of the organisms causing tinea versicolor (*M. furfur*), trichomycosis axillaris (*No. tenuis*), and erythrasma (*No. minutissima*). The second group of fungi are the dermatophytes and contain those fungi that invade the layers of the skin but not the deeper tissues. It is this group of fungi that will represent 65% to 75% of the mycotic infections studied by the technologist, and it is this group that possesses most of the deficiencies in growth factors.

Three genera make up the dermatophytes: *Microsporum, Epidermophyton,* and *Trichophyton.* The first of these contains three species of which only two are important in this country: *M. audouini* and *M. canis* (syn. *M. felineum*). *Epidermophyton* contains only one species: *E. floccosum* (syn. *E. inguinale; Trichophyton cruris*). The *Trichophyton* genus contains about ten species of which seven may be considered important in this country. This genus contains many synonyms for each species, all of which are used by writers as they see fit. Conant and Georg have attempted to standardize the nomenclature so that order may be brought to this particular field. The terminology used in this paper is that of Conant and his associates (1). Following is a list of the common species of *Trichophyton* and a few of their synonyms:

T. mentagrophytes syn. *T. gypseum, T. interdigitale, Achorion Quinckeanum*
T. rubrum syn. *T. purpureum, T. marginatum, Epidermophyton rubrum*
T. tonsurans syn. *T. epilans, T. sabouraudi, T. crateriforme, T. sulfureum*
T. Schoenleini syn. *Achorion Schoenleini*
T. violaceum syn. *Achorion violaceum, T. glabrum*
T. verrucosum syn. *T. album, T. ochraceum, T. discoides*[1]
T. Megnini syn. *T. roseum, T. rosaceum*
T. gallinae syn. *Achorion gallinae, M. gallinae*

MICROSPORUM: The differentiation of the *Microsporum* from the *Trichophyton* is easily done microscopically; the identification of *M. audouini* and *M. canis* is done macroscopically as well as microscopically and usually without much difficulty. Microscopically the Microsporum species usually have microconidia (small conidia of a fungus, a conidium being an asexual spore borne on special structures, e.g. conidiophores) and macroconidia (the larger and generally more diagnostic conidium of a fungus) which are quite different from those of species of Trichophyton. On Sabouraud's medium[2] *M. audouini* will have little aerial mycelium with the reverse of the colony usually being a reddish-brown to a rusty orange color; microscopically it may have a rare abortive macroconidium and occasionally a

[1] Georg has classified this as *T. faviforme* var. *album; T. faviforme* var. *discoides, T. faviforme* var. *ochraceum.*

few microconidia on primary isolation. As a rule *M. audouini* will possess no spores. *M. canis*, when cultured on Sabouraud's medium, usually has a great deal of white, cottony aerial mycelium with the reverse of the colony being a canary yellow to a yellow orange. Microscopically it will have a few microconidia and many characteristic macroconidia that are large, spindle-shaped, multiseptate with rough, thick walls. There are times, however, when *M. canis* will closely resemble *M. audouini* and it is then that the technologist will need a differentiating medium. There are two that can be used in the diagnostic laboratory: rice medium*[2] and honey yeast extract agar* (3). If *M. canis* is subcultured to rice medium it will grow over the surface of the granules but *M. audouini* will not grow on rice medium. These cultures should be kept at room temperature for ten to fifteen days. *M. audouini* will develop its characteristic macroconidia within ten to fifteen days if it is cultured on honey yeast extract agar.

EPIDERMOPHYTON: *Epidermophyton floccosum* is very characteristic grossly when freshly isolated on Sabouraud's medium and little difficulty exists for the technologist in its identification. The colony is velvety to powdery and is a beautiful greenish-yellow color. The reverse of the colony may possess a yellowish to greenish-yellow pigment but this is not characteristic. Microscopically the culture never produces microconidia but does possess many characteristic macroconidia that are smooth, thin walled, with three to five septa, and a blunt end; because of the morphology of these conidia they are sometimes referred to as "snow-shoe" in shape. Although little study has been done to date on the nutritional requirements of this organism there is little doubt that it has certain deficiencies, for it very quickly becomes pleomorphic developing sterile, white cottony mycelium after a few subcultures. It is impossible to keep this fungus in a stock culture collection.

TRICHOPHYTON: It is when the medical technologist attempts to identify species of Trichophyton that the headaches may arise. There are those who would call the identification of these species purely academic since therapy for all is about the same; such is not the case. It is important for the physician to know whether *T. mentagrophytes* or *T. rubrum* is the etiological agent in a case of tinea pedis since the latter is far more resistant to therapy and may give rise to a persistent infection. On the other hand, *T. mentagrophytes* will give rise to an "id" reaction in the patient more readily than *T. rubrum*. This allergic reaction is most frequently manifested as vesicular lesions on the hands, and may result if too vigorous treatment is given to the patient.

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[2] Unless otherwise stated, Sabouraud's medium as referred to in this paper is Sabouraud's dextrose agar, formula in appendix.

* Appendix.

Trichophyton verrucosum is one of the chief etiological agents of the deep type of tinea barbae, giving rise to follicular pustules resulting in abscesses and nodular lesions. These cases are frequently seen in men who come in contact with cattle. Some of these patients are seen in either the medical or surgical clinics for treatment of sycosis vulgaris, a bacterial infection; it is only after a potassium hydroxide preparation and/or a culture is done that many of these cases are recognized as being mycotic in nature. *T. verrucosum* must be differentiated from *T. Schoenleini*, the cause of favus, a serious form of tinea capitis resulting in destruction of hair follicules and, thus, permanent alopecia. *T. violaceum* closely resembles *T. Schoenleini* and is also an etiological agent of favus. *T. Megnini* and *T. gallinae* are etiological agents of tinea capitis and tinea pedis and require care to differentiate them. Fortunately, *T. gallinae* has been seen rarely in this country.

Last but not least is *T. tonsurans*, a more common etiological agent of tinea capitis. Mycologists and physicians have been alerted by the United States Public Health Service to this fungus since there is reason to believe that it may give rise to epidemics of tinea capitis as *Microsporum audouini* has done in the past. Should this happen a more serious problem will exist, for only children, with rare exception, are infected with *M. audouini*, but *T. tonsurans* has no preference as far as age is concerned.

IDENTIFICATION OF TRICHOPHYTON: Microscopically it is impossible to differentiate *T. mentagrophytes* from *T. rubrum;* grossly not all strains of *T. rubrum* will develop its characteristic red pigment on Sabouraud's medium, and conversely, *T. mentagrophytes* will occasionally have a reddish pigment when isolated on this medium. Bocabo and Benham (4) have shown that *Trichophyton mentagrophytes*, when cultured on 1% dextrose corn meal agar,* will not develop any red pigment while over 99% of the strains of *T. rubrum* will become pigmented. It is this method that most medical mycologists use to confirm identification of these two species. Benham (5) has found that *T. rubrum*, when grown on Difco Blood Agar Base,* will produce macroconidia that will also assist in the identification of this species. There will be little difficulty in identifying *T. mentagrophytes* and *T. rubrum* if the medical technologist will keep these two media near at hand.

When cultured on Sabouraud's medium, *T. verrucosum* is a slow growing fungus, developing deep folds in its surface growth, usually glabrous with some isolates producing a fine white surface down; color may vary from white to yellowish brown. Microscopically no spores are seen. Georg (6) has shown this fungus to have a thiamine deficiency and noted that when isolated on a vitamin enriched me-

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* Appendix.

[3] *T. Schoenleini, T. concentricum, T. ferrugineum, T. violaceum.*

dium* and incubated at 37 C large numbers of microconidia and macroconidia are produced. This is the only dermatophyte that requires 37 C incubation for primary isolation; once the fungus has been isolated, however, it may be readily cultured at room temperature.

There is no medium, unfortunately, that will assist us in identifying *T. Schoenleini*. When this fungus is cultured on rice medium it will develop microconidia but these are not characteristic of this species and cannot, therefore, be used for identification. The medical technologist usually can put this into the faviform group[3] but, should she be unable to go further, must send it to a mycologist for final identification.

Trichophyton violaceum grows poorly on Sabouraud's medium. Georg (7) has been able to get this fungus to develop characteristic macroconidia on trypticase thiamine-dextrose agar.* These macroconidia are elongated clavate, having 2-5 cells, are small and thin walled and are hyaline in color.

T. Megnini and *T. gallinae* are two fungi resembling each other very closely; they produce a rose to violet color on Sabouraud's medium, produce some microconidia but no macroconidia. They are easily differentiated. *T. megnini* grows well on trypticase dextrose agar, producing many small, pyriform microconidia and characteristic slightly clavate macroconidia, having 2-8 cells, thin and smooth walled, and measuring 10-35 mu by 3-6 mu (8). On Sabouraud's medium *T. Megnini* gradually turns from a white to a deep rose color with the reverse of the colony becoming a deep red; the pigment does not spread beyond the colony. *T. gallinae*, on the other hand, is not stimulated by trypticase and possesses no special growth requirements. Its microconidia resemble those of *T. Megnini* but its macroconidia are characteristic, being numerous, clavate and having 2-10 cells measuring 15-50 mu by 6-8 mu; the walls are thick and smooth. On Sabouraud's medium the colony is generally white often with light pink areas. The reverse of the colony is a light pink color with diffusion of the pigment throughout the agar.

T. tonsurans is another of the dermatophytes that does not require special growth factors; it grows well on Sabouraud's medium, developing a heaped and folded colony that is powdery in appearance, usually varying from whitish tan to shades of yellow. Microscopically it can be distinguished from *T. verrucosum* by its innumerable characteristic pleomorphic, pyriform to clavate microconidia, some of which are two celled. Macroconidia are rare and not used for identification.

IDENTIFICATION OF FUNGI GIVING RISE TO SYSTEMIC INFECTIONS: The identification of these fungi are much simpler since the cultures either have characteristic micro-

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* Appendix.

scopic appearances or else will develop typical structures when injected into animals. It is true, however, that there are certain media available that greatly facilitate the isolation of these fungi and all medical technologists should know of these and have them available.

With few exceptions isolation of the fungi giving rise to subcutaneous and systemic infections can be isolated and cultured on Sabouraud's medium and blood agar. *Sporotrichum Schencki* is a biphasic fungus, e.g. mold-like at room temperature and yeast-like when cultured under proper conditions at 37 C and when seen in tissues. It grows easily and rapidly on Sabouraud's medium at room temperature but it is difficult to obtain and keep the yeast-form growing on this medium. Campbell (9) found that Francis' glucose cystine blood agar* was an excellent medium for the isolating and culturing of *S. Schencki* in the yeast form. The filamentous stage, however, is used for the identification of this fungus.

When the technologist is requested to culture material for *Histoplasma capsulatum*, Difco brain heart infusion glucose blood agar* is used in conjunction with Sabouraud's medium. Howell (10) and others have shown that a higher percentage of cultures positive for *H. capsulatum* are obtained if material is streaked on the surface of brain heart dextrose blood agar[4] and incubated at room temperature. It is necessary to subculture any growth obtained on this medium to Sabouraud's medium for the development of the characteristic tuberculated chlamydospores.

Cryptococcus neoformans and *Blastomyces dermatitidis* grow well on Sabouraud's medium and blood agar. It is usually wise to add penicillin and streptomycin[5] to these media to cut down growth of bacteria. *Nocardia asteroides* will grow well on Sabouraud's medium at room temperature and *Actinomyces bovis* grows on blood agar or brain heart infusion blood agar at 37 C under reduced oxygen, e.g., a candle jar. Care must be taken, however, that no antibiotics are added to these media since all the actinomycetes are sensitive to these compounds.

Georg and her associates (11) have found it much easier to isolate *Coccidioides immitis* from heavily contaminated materials by adding penicillin and streptomycin and 0.5 mg per ml of Actidione[6] to the medium. Actidione reduces the growth of air borne fungi on medium and keeps those that do grow from spreading over the entire plate. This compound can be added to medium

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* Appendix.

[4] Blood agar usually contains 2%-5% blood, either human or rabbit.

[5] Unless otherwise indicated, 20 U of penicillin per ml and 40 U of streptomycin are added to these media.

[6] Actidione, Upjohn Company, Kalamazoo, Michigan. A technical grade can be obtained for one fourth the price of the therapeutic compound.

used for the isolation of dermatophytes and other fungi pathogenic for man with one exception—*C. neoformans* is sensitive to actidione and for this reason cannot be isolated on media containing it.

This paper would not be complete unless a few words were said about the isolation of the Aspergilli and Penicillia. The incidence of penicilliosis is very rare and should not serve as a problem to the medical technologist. Aspergillosis, however, is far more common and any medical technologist doing mycologic work can expect to isolate a pathogenic species from nails, skin, sputum and tissues. The Aspergilli grow well and quickly on Sabouraud's medium to which penicillin and streptomycin have been added but not Actidione. However, the key (12) for the identification of the species of this genus is based on the growth characteristics as seen on Czapek-Dox medium.* It is not a difficult thing for a technologist to identify a species of Aspergillus if he carefully reads the introductory chapters of Thom and Raper's key to the Aspergilli. The author can assure the technologist that he will get pleasure out of attempting such a classification, for the Aspergilli are beautiful fungi both grossly and microscopically. Since the age of antibiotics and steroids it would appear that the incidence of secondary infections due to the Aspergilli as well as to the Candida are on the increase. Whether this is actually true or whether the medical profession is just becoming more acutely aware of fungi as etiological agents of diseases in man will not be argued here.

The last group of fungi to be discussed are those that are yeast-like when cultured at room temperature as well as at 37 C. This includes the species of Candida, Cryptococci and Saccharomyces. Certain species of the first two are pathogenic to man but the Saccharomyces do not contain any species pathogenic to man but are frequently contaminants of tissue to be cultured and for that reason must be differentiated from the Candida and Cryptococci. The Candida contain seven species of which one is considered pathogenic to man, *C. albicans*, although other species have been affiliated with pathologic lesions (13). There are many species of Cryptococci but only one is pathogenic for man—*C. neoformans*. When Candida is inoculated to corn meal agar* without dextrose or a medium made of a derivative of corn meal* of which there are two (15, 16), by cutting deeply into the agar with a straight wire, and then covering the cuts with a sterile coverslip, mycelium bearing clusters of budding cells will be seen to develop. This identifies the fungus as a member of the Candida genus but the technologist must turn to a key (14) for the final differentiation of the species. Cryptococci and Saccharomyces will not develop mycelium in this medium but will continue to

* Appendix.

bud. To differentiate these two groups it is necessary to induce production of ascospores by the Saccharomyces which the Cryptococcus do not produce. Sporulation of yeasts can be brought about by culturing them on McKelvey's medium.* Having identified the organism as a Cryptococcus species or as a member of the Saccharomyces genus it will then be necessary to turn to references (1, 17) of standard works for further assistance.

The author wishes to thank the Nancy Sayles Day Foundation for assistance in preparing this material.

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APPENDIX

1. Sabouraud's medium:

Glucose	40 grams
Agar	35 grams
Neopeptone	10 grams
Distilled water	1000 ml

Place this mixture in the autoclave or Arnold to melt agar, then filter through cotton gauze and tube. Autoclave at 15 pounds for 15 minutes and slant. It is not necessary to pH this medium.

2. Rice medium for Microsporum species:

Polished rice	8 grams
Water, distilled	25 ml

Place the rice and water in 125 ml Erlenmeyer flask and autoclave for 15 minutes at 15 pounds pressure.

3. Honey yeast-extract agar: for *M. audouini:*

Commercial honey	60 grams
Peptone, Difco	10 grams
Agar	20 grams
Water, distilled	1000 ml

Make up a 5% solution of Bacto-dehydrated yeast extract and sterilize by running through a Berkefeld filter. When the above medium has cooled down to 45 C, add the yeast extract aseptically to give a final concentration of 5 mg per ml of medium.

4. Corn Meal Agar (dehydrated corn meal has not been satisfactory for the development of chlamydospores of *C. albicans*):

Yellow corn meal agar	62.5 grams
Water, distilled	1500 ml.

Heat this mixture at 60 C for one hour; filter through coarse filter paper and return volume to 1500 ml. Add 19 grams of agar to this mixture, heat in an Arnold for one hour, filter through cotton. To differentiate *T. rubrum* and *T. mentagrophytes* add enough dextrose to give a concentration of 1%, tube and sterilize for 25 minutes at 15 pounds. The pH should be between 5.5 and 7.5. Too acid a pH will cause *T. rubrum* to develop only a cream to yellowish pigment.

5. Difco Blood Agar Base:

Beef heart, infusion from	500 grams
Bacto-tryptose	10 grams
Sodium chloride	5 grams
Agar	15 grams

The dehydrated medium can be obtained from Difco; no adjustment of pH is necessary.

6. Thiamine agar for *T. verrucosum:*

Thiamine hydrochloride, 10 mg per liter, is added to Difco blood agar base. The medium should be adjusted to a pH 6.8, and autoclaved at 121 C for 10 minutes. If desired, 20 U of penicillin and 40 U of streptomycin per ml may be added just before use of this medium. This is done by melting the medium and allowing it to cool to 45 C before adding the antibiotics.

7. Trypticase-thiamine dextrose agar for *T. Violaceum:*

Baltimore Biological Laboratories Trypticase agarto which 5% dextrose has been added and 10 mg per liter of thiamine hydrochloride.

8. Francis' dextrose cystine blood agar for *Sporotrichum Schencki:*

Add 1 gram of powdered cystine to 1,000 ml of sterile infusion agar and heat in flowing steam in an Arnold sterilizer for two hours. Place in a 56 C water bath overnight. The next morning add 60 ml of sterile blood and heat to 60 C for two hours. Add 20 ml of a 50 per cent sterile solution of glucose and distribute aseptically into test tubes.

(Schaub, I. F., and Foley, M. K.: Diagnostic Bacteriology, Fourth Edition, St. Louis, The C. V. Mosby Company, 1952.)

9. Difco Brain Heart Infusion Agar for Histoplasma capsulatum:

This can be obtained in the dehydrated from the Difco Laboratories, Detroit, Michigan. Penicillin and streptomycin may be added before use.

10. Czapek-Dox medium for Aspergillus species:

$NaNO_3$	2 grams
KH_2PO_4	1 gram
KCl	0.5 gram
$MgSO_4 \cdot 7H_2O$	0.5 gram
$FeSO_4 \cdot 7H_2O$	0.02 gram
Glucose	50.0 grams
Distilled water	1000.0 ml

No adjustment of the pH is needed.

11. Zein medium (15) for the development of chlamydospores of *C. albicans:*

Zein (Nutritional Biochemical Corporation, Cleveland 28, Ohio). 40 grams to which 1000 ml of distilled water is added. Heat this in a waterbath for 1 hour at 60 C. **Note:** As soon as this mixture reaches 60 C the Zein will become one large, sticky, insoluble ball; do not become alarmed but continue the heating process. We have found it advantageous to use a 2000 ml beaker for making this medium since it is almost impossible to clean an Erlenmeyer flask following its use in making this medium. Filter this mixture through gauze and coarse filter paper and make up to volume. Add 15 grams of agar and steam in an Arnold for ½ hour; place in flasks or tubes and autoclave at 121 C for 15 minutes. It is not necessary to pH this medium.

12. Polysaccharide medium for *Candida albicans:*

Basal medium:

$NH_4 \cdot SO_4$	1.0 gram
KH_2PO_4	1.0 gram
Biotin	5.0 grams
Agar	15.0 grams
Trypan blue	0.1 gram
Distilled water to make 1000 ml.	

Sterilize purified polysaccharide separately by autoclaving; add 2 grams per 100 ml of this polysaccharide aspectically to the sterilized basal medium. **Note:** Glycogen (Pfanstiehl) as obtained commercially needs no further purification but is expensive. Should you desire to use corn starch it will be necessary to remove reducing sugars which are frequently present, refer to the work of Nickerson and Mankowski (16).

13. McKelvey's medium for sporulation of yeasts:

Weak carrot infusion (150 grams of chopped carrots to one liter of tap water). Agar 15 grams. Melt together; then add 3.5 grams of anhydrous calcium sulfate, mix, tube, autoclave at 121 C for 15 minutes. No adjustment of pH is necessary.

METHODS FOR THE ISOLATION OF PATHOGENIC FUNGI FROM CLINICAL MATERIAL*

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The isolation of molds and yeasts pathogenic for man from clinical material and their subsequent identification is not one of the primary tasks of the diagnostic laboratory, but requests for this service come with enough frequency to be somewhat disconcerting to the technologist. The usual procedure is to plant any specimen upon so-called "Sabouraud medium"[1] and wait several days to see whether or not a fungus appears. If the fungus does not appear, the troubles of the technologist are ended; but should the aerial hyphae appear the technologist must discriminate between the few pathogens and the numerous saprophytes, while if a moist yeasty growth appears she must make a similar decision for this type of fungus.

Perhaps we should now consider some of these pathogenic fungi and determine how they may be identified and how they may be distinguished from the saprophytic fungi. Our problem is not simplified by the fact that pathogenic fungi do occur as saprophytes in soil and in rotting wood. Many fungi grown upon artificial culture media produce only sterile hyphae and yield none of the morphological structures which are useful in identification. It is also disconcerting to realize that so great is the number of saprophytes that it may require a lifetime for a student to become familiar with the species in a single genus. However, technologists have come to realize that the saprophytes will produce an abundant growth of aerial hyphae in three or four days at 25 C, and that shortly after these have appeared the formation of conidia will give a powdery appearance to the mycelium, and that as these conidia ripen a characteristic color (which may be black, green, pink, yellow or white) will develop. Those pathogens which can produce aerial hyphae, may do so after one or two weeks incubation at either 20, 25 or 37 C. In the pathogens it is the hyphae growing in the substrate that usually produce a pigment which diffuses somewhat into the agar and is best seen on the reverse side of growth. Only a limited number of yeasts and molds produce lesions in man, and the mycologist who lives in the temperate zone will not be confronted with all of these. A list of the more common species found in the United States follows.

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I. The Dermatophytes. Those fungi which attack the keratin in skin, hair and nails.

Genus *Microsporum*	human group *M. audouini*
	animal group *M. canis*
Tricophyton	gypseum group *T. mentagrophytes*
	rubrum group *T. rubrum*
	T. tonsurans
	faviforme group *T. faviforme*
	crateriform group *T. schoenleini*
Epidermophyton	*E. floccosum*

II. Those fungi which cause systemic diseases.

Blastomyces dermatitidis
Coccidioides immitis
Cryptococcus neoformans
Histoplasma capsulatum
Sporotrichum schenckii

III. Intermediate group. Those fungi which may attack epidermis and nails, or may cause systemic disease.

Candida albicans

The principal difficulties in laboratory diagnosis are to secure material free from contamination, to detect the organisms in mounts made directly from the lesions, and to induce growth on laboratory media. The tools needed to secure the clinical material consist of scalpels for scraping skin and ulcer margins, epilating forceps for removal of hairs, cuticle scissors for unroofing vesicles, nail clippers, sterile 5 ml syringes with 18 or 20 gauge needles for aspiration of abscesses (Moss and McQuown[2]). A lamp which emits filtered ultraviolet light is essential for securing samples of infected hairs. Margarot and Devese[3] made the observation that hairs infected with dermatophytes would usually fluoresce and that cultures of the causative organisms would also give fluorescence if examined with ultraviolet light which had been filtered through Woods' nickel oxide glass. The lamp does not need to be an expensive model, but it should be portable and built so that it is easy to direct the beams over skin surfaces of the body or over the hair on the head.

A generous sample of the infected hairs should be epilated and placed in a sterile petri dish. From the margins of skin lesions scrapings should be made and transferred to a sterile petri dish.

A time honored method for the examination of this material is to clear the skin scales or the hair by mounting them in 10% sodium hydroxide on a microscope slide under the protection of a cover slip. The clearing time may be hastened by the application of gentle heat.

Ordinary bacterial stains are of little value for examination, but Moss and McQuown[2] recommend the Hotchkiss[4]-McManus[5]

stain, which is one of the most recent modifications of periodic acid leuco-fuchsin techniques. The fungi stain a brilliant magenta.

Periodic Acid-Fuchsin Stain of Hotchkiss-McManus

1. Spread skin scrapings over a slide coated with Mayer's albumen.
2. Immerse in 95% ethyl alcohol for 1 minute.
3. Immerse in 5% aqueous solution periodic acid for 5 minutes.
4. Immerse for 2 minutes in
   Basic fuchsin ..... 0.1 gram
   95% ethyl alcohol..... 5.0 ml
5. Rinse in tap water
6. Immerse for 10 minutes in
   Zinc hydrosulfite ..... 1.0 gram
   Tartaric acid ..... 0.5 gram
   Water .....100.0 ml
7. Rinse in tap water.
8. Immerse in light green 5 seconds.
   Light green ..... 1.0 gram
   Glacial acetic acid..... .25 ml
   80% ethyl alcohol.....100.0 ml
9. For temporary mount use glycerol.
10. For permanent mount dehydrate in 95%, absolute ethyl alcohol, two washes of xylol and mount in clarite.

For the direct examination of pus, bone marrow or liver biopsy material, Wright's blood stain or Giemsa's stain may be used. With either of these stains the character of the exudate can be identified, while the branching filaments of *Actinomyces israeli* can be seen (associated with abundant neutrophilic polymorphonuclear exudate) or the yeast phase of *Histoplasma capsulatum* can be identified (free or in the cytoplasm of monocytes or histiocytes). The budding yeast phase of *Blastomyces dermatitidis* can be seen (associated with an abundance of polymorphonuclear leukocytes) but this fungus cannot be identified positively without further culture.

A useful Giemsa stain formula has been reported by Wilcox:[6]

Azure B ..... 250 mg.
Azure A ..... 55 mg.
Methylene blue ..... 310 mg.
Eosin Y ..... 537 mg.

These dyes are mixed to a paste with 50 ml. of neutral C.P. glycerol and then dissolved in 50 ml. of absolute methyl alcohol which is acetone free. The stock solution should be kept tightly stoppered because each of the solvents is hydroscopic.

In use it is desired to have a phosphate buffer of pH 7.2.

M/15 Na_2HPO_4 ..... 7.20 ml
M/15 KH_2PO_4 ..... 2.80 ml
Distilled water .....90.0 ml

The Giemsa method is a little more trouble than the Wright

method, but it gives permanent mounts which retain the stain for a longer period, and it seems to give more elegant detail.

1. Prepare a thin film of pus or tissue on a microscope slide.
2. Immerse in absolute methyl alcohol in a Coplin jar for 1 minute.
3. Prepare a 1 or 2% solution of the stock Giemsa, using the buffer as a diluent, and place in a Coplin jar for 30 minutes.
4. To avoid a precipitate on the slide, flush out the dye from the Coplin jar by holding it under the tap and adding running water until all traces of dye have been washed out. Dry.
5. Examine with the oil immersion lens.

With Giemsa or Wright's stain *Actinomyces israeli* stains blue; the *H. capsulatum* appears as an avoid body which has an unstained halo, a definite cell wall, and a dark blue nucleus may be identified at one side of the cell. Several of these cells may be packed in a macrophage from the host.

For pus from Blastomycosis infections the sodium hydroxide mount is to be preferred because this will dissolve the abundant polymorphonuclear leukocytes and free the alkali resistant yeast cells for observation.

Exudate from *Crytococcus neoformans* (the cause of Gilchrist's disease or Torulosis) does not contain polymorphonuclear leukocytes. The organism is a spherical budding yeast-like cell with an enormous capsule. It is best demonstrated in a preparation with an India ink background. Do not substitute nigrosin for the India ink or the capsule will appear only as a narrow band.

A general mounting fluid for the preparation of temporary mounts from pure cultures of yeast or molds is the Lacto-phenol-cotton-blue reagent. Ainsworth and Bisby[7] give credit to Amman, 1896, for the preparation of this reagent. (See also Dodge,[8] page 67.) It does not seem to be quite so well known to general technologists in American laboratories and the formula is included in this report.

Lacto-phenol-cotton-blue mounting fluid.

Phenol crystals	20.0 grams
Lactic acid	20.0 grams
Glycerol	40.0 grams
Distilled water.....	20.0 grams

Dissolve these ingredients by heating gently under a hot water tap and add 0.05 grams of cotton blue.

Each of these methods may be used for the microscopic examination of material secured from patients and of cultures which have been isolated from this material. A diagnosis may be made from the microscopic examination alone in Coccidioidosis, Histoplasmosis and Cryptococcosis (Torulosis) while a tentative diagnosis may be given in infections with *Actinomyces israeli* and

Blastomyces dermatitidis. Fungi cannot be identified in skin scales or infected hairs. Many of the fungi must be cultured and studied to secure a species diagnosis.

Methods for the culture of *Coccidioides immitis* will not be included in this paper. Cultures of this fungus are dangerous and several laboratory infections have been reported. I have received information from other laboratory workers concerning the safety measures which they follow, but it would seem desirable to have first hand information and personal experience in handling the fungus in the laboratory if directions for its culture were to be included.

The Sabouraud medium in such common use today is essentially a 3% glucose, 1% peptone medium adjusted to pH 6.6 and solidified with 2% agar. It is not an especially good medium for the growth of fungi, but it is an even worse medium for the growth of bacteria, and since the specimens with which technicians must work are often grossly contaminated, this routine use of Sabouraud medium does serve a purpose.

A much better medium to delay the growth of bacteria and to initiate the growth of pathogenic fungi is Littman's medium.[9] This contains 1% peptone, 1% glucose, 2% agar, 1.5% oxgall and .001% crystal violet. Previous to use it may be fortified with streptomycin to give a content of 30 units per ml. Some strains of pathogenic fungi will not grow upon either Sabouraud or Littman medium.

The use of actidione in a 4% glucose, 1% peptone agar has been described by Fuentes et al.[10] Solutions of actidione are added to the sterilized glucose agar to give a concentration of 0.1 mg of actidione per ml. It was found that this medium had some inhibitive effect on saprophytic fungi.

Leise and James[11] used an alkaline pH to suppress these saprophytes, since they found that dermatophytes were tolerant over a wide pH range (from 3.0 to 10.5) but that the saprophytes were less tolerant of the high pH values.

If it is possible to secure specimens which are not contaminated, blood agar is the medium of choice for the initiation of the growth of many pathogenic fungi. Raubitschek[12] used Difco stock culture agar enriched with 5% human blood and secured growth with 14 specimens out of 24, although with a maltose agar medium he secured only 6 cultures from the same 24 specimens.

The cultures from clinical material should be saved for about four weeks and prepared in duplicate so that incubation temperatures of 25 C and 37 C may be employed. It is always well to use generous inocula when working with pathogenic fungi, whether the purpose is to isolate or to transfer. The streak method of inoculation is not very successful with pathogenic fungi; instead, bits of tissue or bits of agar plus culture are

patted into the medium in spots, using a stiff loop. The cultures must be sealed to prevent drying. *Candida albicans* and *Cryptococcus neoformans* grow most rapidly and produce their characteristic colonies in 48 hours. *Candida* colonies are white and pasty while the *Cryptococcus* colonies are extremely mucoid.

When the primary isolation has been made the cultures may be transferred to media designed to promote characteristic growth or particular morphological features which aid in identification. These features include the formation of a mycelium with hyphae, the suppression of hyphae and formation of a characteristic yeast phase, or the production of specific microconidia and macroconidia. In general we have learned that synthetic or "starvation" media, lower temperatures and sometimes microaerophilic conditions favor the development of hyphae, while enriched media and a 37 C temperature favor the yeast phase. For some of the dermatophytes the particular food accessory factors which favor macroconidia development have been discovered.

After a growth has been obtained from the primary inoculum, particles of agar and culture from the periphery of the growth are obtained and transferred to slants of medium similar to that which provided the primary culture. The next problem is the identification of the culture.

Micro-cultures may be made by mounting inoculated medium on sterile slides under sterile cover glasses and incubating the preparation in a petri dish containing a piece of moistened filter paper. This serves as a moist chamber. The micro-cultures are especially susceptible to contamination, but when successful they often afford a preparation in which the characteristic growth pattern can be readily observed.

As information in regard to the effect of the substrate upon the quantity and quality of growth has increased, reliance upon culture media to help in the identification of unknown cultures has increased.

Those pathogenic fungi which are yeast-like and normally grow in a smooth pasty colony must be distinguished from the saprophytic sugar fermenting yeasts which they resemble. A culture which produces abundant acid and gas in glucose broth after 24 hours incubation and which produces ascospores on carrot plug medium after 3 or 4 days is doubtless a member of the *Saccharomyces*. The pathogenic *Cryptococcus neoformans* and *Candida albicans* do not produce this abundant gas, and ascospore formation has not been reported for these organisms. Extended mycelial growth in septate hyphae cannot be produced with the *Saccharomyces* or with the *Cryptococcus*, although elongated germ tubes have been photographed (Conant)[13] and abortive mycelium formation with the *Saccharomyces* (Scherr)[14] (Scherr and Weaver).[15] To produce the characteristic septate hyphae with

the clusters of blastospores which identify *Candida albicans*, a well tested medium has been prepared from corn meal. Yellow corn meal (40 g) is allowed to simmer in a liter of water at 65 C for one hour. This is filtered through gauze and filter paper and, after the volume has been restored by the addition of water, 20 grams of agar is dissolved and the medium dispensed and sterilized in the autoclave.

The medium which has been used to produce the yeast phase is some modification of the medium which Francis used for the cultivation of *Pasteurella tularense*. One such modification is given here. It has been used with success to obtain the yeast phase of *Histoplasma capsulatum, Blastomyces dermatitidis, B. brasiliensis* and *Sporotrichum Schenkii*. Use one liter of double strength meat infusion and dissolve in it 10 grams peptone, 10 grams glucose, 5 grams sodium chloride and 20 grams of agar. Determine the amount of sodium hydroxide solution needed to adjust the reaction to pH 7.6-7.8 and dissolve in this 1.0 gram of cystine or cystine hydrochloride. Mix the two solutions and dispense in tubes. Sterilize in the autoclave. Before use melt and cool the medium and add to each tube about 8% sterile blood. (Communicable Disease Center, Atlanta, Georgia[16]).

Blastomyces dermatitidis is sensitive to temperature changes; on blood agar at 20 C a white filamentous mycelium develops, while on the same medium at 37 C short septate hyphae and budding cells are found. This fungus also grows on Sabouraud medium, but the culture seems to remain viable for longer periods on the blood medium.

Histoplasma capsulatum readily reverts to the white filamentous type of growth, but it seems to be more difficult to maintain this mold in the yeast phase. Titsworth and Grunberg[17] suggested the use of an egg-potato medium commonly used for growing *Mycobacterium tuberculosis* and claim that on this medium the yeast phase is found after one or two transfers at 37 C. Their medium was made in two parts. A mixture of 200 grams of peeled and ground potatoes, plus 60 ml of glycerol, 0.2 gm citric acid and 5.0 gm bactohemoglobin per liter was cooked in the autoclave and filtered through gauze. This was then sterilized in the autoclave. When cool it was added to a second mixture of eggs prepared aseptically. One whole egg to 11 yolks were used. Equal parts of the two mixtures were combined and to the whole was added 10 ml of 10% Congo Red per liter. The mixture was tubed and inspissated.

It is sometimes of interest to the technologist to produce a reversion to the yeast phase, but with *Histoplasma capsulatum* it is the production of tuberculate spores which is the most striking morphological feature. These form in the hyphal phase and after the primary culture has been secured *H. capsulatum* grows readily upon either glucose peptone agar or blood agar. The optimum pH for this fungus was found by Cross[18] to be 7.2, although it was tolerant of a wide

range of pH values. In the demonstration of the tuberculate spores an extended period of incubation has been found necessary. Very recently Mrs. Pattie in our laboratory has discovered them after 6 days incubation at room temperature in a micro-culture mount.

The newer knowledge of the nutrition of pathogen molds (Georg,[19] Benham[20]) has been especially helpful to the technician who must identify the dermatophytes. The polished rice medium of Conant[21] showed that, while *Microsporum gypseum* and *M. canis* (primary animal pathogens) grew upon this medium and produced characteristic macroconidia, *M. audouini* was a deficient organism and could not grow upon it. The medium is very simple; a little polished rice is put into an Erlenmeyer flask, moistened with distilled water, and autoclaved. No one factor has been discovered which stimulates the production of macroconidia in all dermatophytes or even in all strains of a single species. Hazen[22] reported good results for *M. audouini* with honey agar enriched with yeast extract. Georg[23] showed that the growth of the faviform *Tricophyton* is stimulated by enriched media. Georg[24] demonstrated macroconidia formation for the first time in cultures of *T. violaceum* using a thiamin enriched casein agar. Perhaps the clinical technician may find that agar enriched with fresh blood will provide the necessary growth factors and prove to be an all purpose medium for the production of macroconidia.

About fifteen years ago the most common dermatophyte was the animal species, *M. canis;* in 1943 infections with *M. audouini* began to appear frequently in children. This was true in New York City in 1943, and after I came to Kentucky in 1944, Dr. Thompson and I isolated several strains of the *M. audouini.* In 1952 Georg[25] reported the appearance of another fungus, as she found that infections due to *Tricophyton tonsurans* were increasing, particularly in Texas and other southwestern states. Georg stated that with this fungus there is little fluorescence of the scalp lesions, that the infected hairs tend to bread off at the surface of the scalp, and that on direct examination the spores may be seen packed in chains in the interior of the infected hairs.

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LYMPHOCYTES AND LYMPHOCYTOSES*

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Lymphocytoses, loosely defined, mean conditions in which there is an increase in the mass of lymphocytes in a given volume of blood, or *precisely* defined it means an increase in the total number of lymphocytes in the circulation blood. These increases may be relative and transient, or absolute and even permanent. The permanent lymphocytosis is more aptly defined as a lymphatic leukemia. The relative and absolute lymphocytoses will be discussed later. Now for an understanding of the lymphocyte itself.

The cellular elements of the blood are for the greater part the red blood cells, yet roughly speaking for every 500 red blood cells there is one white blood cell. If whole blood is centrifuged the hematocrit demonstrates about 50 parts in a hundred red blood cells, and one part in a hundred white blood cells. This latter fraction is so little, and since of lesser specific gravity, rests on the red cell packing in a layer called, becaused of its whiteness, the "buffy coat."

Among the members of the buffy coat is the white blood cell called the lymphocyte. It is sometimes seen outside the blood vessel, particularly in loose connective tissue, such as is easily visualized under the skin, or that which makes up part of the lining of the gastro-intestinal tract, the mucosa. In loose connective tissue the lymphocyte does not remain long but transforms into histiocytes, polyblasts, or plasma cells or passes on into fibroblasts of scar tissue.

In the adult 22-30 per cent of the circulation white cells of the blood are lymphocytes; in infants and children up to 10 years of age 40-70 per cent are lymphocytes.

Lymphocytes are common not only to all higher animals but, unlike red blood cells, their counterparts are to be found in invertebrate animals as well.

Historically the first indirect recognition of a lymphocyte was that made by Virchow, when he observed the presence of white blood cells in leukemia but erroneously regarded the condition as a pyemia. Next in order was that made by Metchnikoff when he recognized the part the white blood cells, the lymphocytes to a minor degree, played in the process of phagocytosis. He observed that many of the phagocytes were white blood cells that had wandered into tissue from the blood stream. It was Ehrlich that eventually made the clear-cut distinction in 1879 of noting that white blood cells were either granulocytes or non-granulocytes. The lymphocyte being a non-granulocyte was thus identified. His study incidentally was made on stained blood smears.

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Embryonically, it is generally conceded the first leukocytes are produced extravascularly in the liver from liver mesenchyme as early as the 15mm. (6-7wk.) stage; and in the spleen and bone marrow at the 9cm. (3mo.) stage, and by the 4th and 5th months are found extensively in the bone marrow. The liver and spleen subside in granulocytic production gradually until birth when scarcely any evidence of granulocytic centers is to be found in these organs. As to non-granulocytes or the lymphocytes (and monocytes) there is still much controversy as to their origin. One thing is certain there are no lymphocytes in the embryo and *hence these earlier leukocytes must be granulocytes.* However, lymphocytes are seen in fetal life when the arterial blood supply to the spleen is complete and arteries have lymphoid sheaths about them there.

The problem of normal regeneration of lymphocytes arose with the concept of separate lymphocytic and myelogenous regenerations as first stated by Ehrlich in 1880. The question is difficult to solve because of conflicting opinions and because there never have been applied to the derivatives in the lymphocytic development, names such as are found in the granulocytic and hemoglobiniferous series. The nearest to these terms are those coined by Sundberg and appearing later in this paper. The controversy applies more to the heteroplastic than homoplastic type of regeneration. The real point in the controversy is whether this heteroplastic generation is limited or not.

As said before lymphocytes are found in all vertebrates. This is one reason why so many think that the lymphocyte is the ancestral stem cell. In amphibia the lymphocytes look like the immature lymphocytes of humans. Extreme unitarians regard lymphocytes of normal blood, normal lymphatic tissue, and normal connective tissue, equivalent no matter where found to the stem cell, for *all* types of blood cells normally. This is not true; the lymphocyte is a polyvalent cell with the potentiality to differentiate to many types of cells only if the proper stimulus is applied. This will be shown later. In the blood stream the lymphocyte is a relatively inactive cell.

Maximow and Bloom spoke of a large lymphoid cell occurring in bone marrow and lymph nodes and in the peripheral blood in leukemias as a "large lymphocyte." Later these very same authors called that same cell "hemocytoblast." Recently it has been shown that the hemocytoblast or large lymphocyte is really the hematopoietic reticular cell, an immature cell limited to marrow, spleen and lymph nodes. The "medium lymphocyte" in Maximow's work is the more recently accepted "reticular lymphocyte" while the "small lymphocyte" is that which is seen normally in peripheral blood.

Downey and Weidenreich in 1912 gave us one theory for the

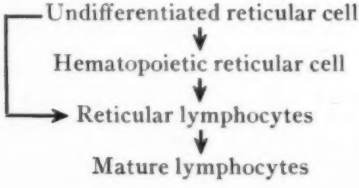
origin of lymphocytes, and this theory is the most modern in cytological development. It remained for Sundberg thirty years later to complete it. According to Downey lymphocytic development is both homoplastic and heteroplastic, normally.

According to Downey, *et al.* lymphocytes normally develop from lymphoid tissue, in the lymph nodes, white pulp areas of the spleen, solitary follicles of the tunica propria, and in loose connective tissue—but this rarely. In these structures the germinal center consists of reticular connective tissue with reticular cells and relatively few fibers. The predominant cells in the tissue are the medium sized lymphocytes (reticular cell) but there are many mitotic figures, less differentiated lymphoid cells (hematopietic reticular cell), more differentiated cells (mature lymphocytes—large in the center, smaller toward the periphery), and some macrophages.

In Sundberg's mind, this type of regeneration which is heteroplastic because it is from fixed and free reticular cells, not only in the germinal center but in areas of diffuse lymphatic tissue is suggestively as follows: The first proposed stage is the undifferentiated reticular cell. This cell may transform either to a "blast" cell which is sometimes designated as the "blast cell of the reticulum," or, as a "hematopoietic reticular cell"; or it may pass directly to cells resembling the reticulum but also bearing resemblance to mature lymphocytes as well. These cells resembling the reticulum and at the same time mature lymphocytes are collectively referred to as "reticular Lymphocytes." No effort is made to designate specific stages of development among the "reticular lymphocytes."

These "reticular lymphocytes" undergo numerous mitoses and the transformed products of their proliferative processes are the mature lymphocytes—the various types which find themselves in the peripheral blood.

Sometimes in this heteroplastic type of lymphocytic regeneration the "blast" stage is skipped and differentiation occurs directly from the reticulum to the "reticular lymphocytes." In summary the following holds:



It is obvious that there is no need of an interposing stage, in other words, no need of a stem cell like the myeloblast. Thus in the heteroplastic regeneration the "reticular lymphocytes" are the intermediary stages through which normal lymphocytes must pass.

The description of the undifferentiated reticular cell is as follows. It has a diameter of about 30 micra. The nucleus is fairly small with respect to the cytoplasm (may fill half the cell), round, oval, or slightly indented. There is a fairly delicate distribution of the chromatin which is not leptochromatic nor continuously net-like as in the myeloblast but only somewhat stippled, for occasionally it is made of comma-shaped particles, strands of complete rings—5 and 6 of these linked and several links lying parallel making small unique areas. There may be small definite clumps sometimes connected to each other by thin strands of chromatin. The general pattern may be called very appropriately "lambskin." The parachromatin is sharply demarcated from the chromatin and at different levels of focus may appear as spheres—very pink staining (with Wright's). Sometimes the chromatin and parachromatin are not well demarcated giving the nucleus a "muddy" appearance. The nuclear membrane appears delicate and indistinct in some cells.

The nucleoli are variable in number, nearly always irregular in size, varying from 1-2 micra to 4-5 micra in diameter and indistinct in outline staining pale blue. Sometimes there are masses of what appear to be confluence of smaller nucleolar material.

The cytoplasm is usually a very abundant liquid, flowing out from the nucleus with seldom a well-defined cell boundary (rather pseudopodic). The cytoplasm consists of a mixture of hyaloplasm and spongioplasm, perhaps more of the former. The mixture may be granular and vacuolated or it may appear foamy and bubbly—in either case it is not homogenous but mottled.

The granulation may be minute azurophilic granules.

The potentialities of this cell are that it may become a macrophage or a hematopoietic reticular cell.

For the description of the other cells in the heteroplastic type of regeneration the following table is constructed, making it possible to compare them with the so-called "stem cells" or myeloblast and lymphoblast.

From the chart below it becomes apparent that the undifferentiated reticular cell rounds up as it becomes the hematopoietic reticular cell, the latter very superficially resembling the myeloblast. In transforming into a reticular lymphocyte the hematopoietic cell retains its relatively abundant cytoplasm but becomes more

basophilic in that component. Most of the mitotic stages in the lymph modes are at the hematopoietic reticular and reticular lymphocytic stages.

Homoplastic regeneration is the development of lymphocytes from pre-existing lymphocytes, the medium-sized lymphocyte being the parent for the large and small. Small, medium and large lymphocytes are interchangeable, the change being accomplished by the loss or gain of fluid. As soon as the lymphocyte enters the peripheral blood further development is inhibited.

LYMPHOID CELLS IN HETEROPLASTIC REGENERATION

Cell Type	Hematopoietic Reticular Cell	Reticular Lymphocyte	Large Lymphocyte	Myeloblast or Lymphoblast
Cell Size Nucleus	23 micra x 19 micra 17 x 14	20 mu x 17 mu 16 x 13	14 mu x 12 mu 11 x 9	16 mu x 15 mu 14 x 13
Nuclear shape	Round or oval	Round or oval	Round or oval	May be irregular
Nuclear pattern	Irregular (superficially resembles myeloblast)	Irregular (looser than that of the myeloblast)	Irregular, more pachychromatic*	Regular, leptochromatic
Chromatin Staining Nature	Light reddish violet Small particles of variable size; short linked chains; strands, granules and rods	Reddish violet Similar to the description on the left but larger masses—triangular and pennant-shaped	Purple Large, angular masses blending with parachromatin in "hill and valley" effect	Light reddish violet fine, sievelike or net-like; sometimes stippled or granular
Parachromatin Amount and nature	Abundant and very distinct	Abundant and more distinct than in myeloblast	Sparse and INDISTINCT	Distinct but not overly abundant
Staining	Pale pink	Pale pink to pale reddish violet	Pale reddish violet to gray-blue	Pale pink
Nucleoli Number Diameter Staining Shape Character	Variable 1–7 micra Pale blue Rounded or irregular Indistinct	Variable 1–7 micra Pale blue to blue Rounded or irregular Indistinct (obliterated by chromatin clumps)	Not visible	Variable 1–3 micra Pale blue Round or oval Indistinct
Nuclear membrane	Fine, distinct	Coarse, less distinct	Coarse, less distinct	Very fine, indistinct
Cytoplasm Staining Character	Gray-blue to colorless Mottled and flaky due to 50:50 ratio of hyaloplasm and spongioplasm	Deep blue to colorless Mottled and flaky; a uniform mixture of hyaloplasm and spongioplasm or a mottled perinuclear zone and hyaline peripheral zone	Pale blue to colorless Usually homogenous	Pale blue Usually homogenous
Vacuoles	Common	Common	Uncommon	Uncommon
Azure granules	Rare	Rare	Uncommon	Common to myeloblast but uncommon to lymphoblast

*Note: These words qualify the appearance of the chromatin: "Leptochromatic" means a fine reticular network and gives a "younger" appearance to the nucleus; "pachychromatic" means thick strands such as in mature lymphocytes; "Amblychromatic" means pale staining, while "trachychromatic" means dark staining of the chromatin.

There are three types of lymphocytes in the peripheral blood—large, medium and small—small in the sense that they are little larger than red blood cells. Here a confusion might arise in terminology for a large lymphocyte of the blood stream may be small as compared with a lymphocyte in the lymph node—the latter may be twice the size of the former. Large lymphocytes—such as are in the lymph nodes and other organs of lymphoid tissue—are not seen in peripheral blood unless a pathological condition exists. "Large" of the circulating blood are "medium" lymphocytes of the tissue.

The description of the peripheral blood lymphocytes is as follows:

Morphology: *Diameter:* In the living preparations 5-8 micra; in dry fixed smears 7-15 micra. Small: 7-9 micra; medium 9-11 micra; large 12-15 micra.

Nucleus: Round, oval or slightly kidney-shaped with coarse chromatin masses—pachyochromatic; and dark staining—trachychromatic. The parachromatin is not well-demarcated from the chromatin—rather a hill-valley blending of the two.

In the *small* lymphocyte the nucleus fills almost the whole of the cell; heavy clumps of chromatin are seen.

In the *medium* lymphocyte the chromatin stains darker while the faint parachromatin stains lighter giving a cloudy effect.

In the *large* lymphocyte the nucleus is large and less clumped with more parachromatin that stains reddish pink but not much demarcation. The nuclus may be eccentric especially in pathology.

Nuclear membrane: Thick, prominent in all three lymphocytes.

Nucleoli can be seen in vitally stained preparations or tissue sections but not in dried fixed smears, except in the smallest if the nuclear membrane is broken.

Cytoplasm: Hyaline, colorless to pale blue (robin's egg blue). In the *small* lymphocyte it appears merely as a rim of pale blue; in the *medium* more than a rim of pale blue, and in the *large* still colorless to pale blue but more abundant and sometimes vacuolated.

Granules: These are non-specific or azurophilic (reddish or reddish-purple) seen in ⅓ of the medium and large sized lymphocytes but seldom in the small. In the large lymphocytes the granules may be spherical and sometimes as large as eosinophilic granules.

Fat droplets: (or more highly refractile bodies) are seen if cells are stained with Soudan III in a small percentage of the lymphocytes and one or two such droplets per cell.

Mitochondria: These are seen if stained with Janus B. green supravitally. Schridde thought young and old lymphocytes could be separated from all other types of blood cells on the basis of mitochondria—they disappearing in the maturing cell, but this feature has not been dependable.

Now for a discussion of abnormal regeneration. This may be:

1. Accelerated normal heteroplastic regeneration as seen in certain leukemoid reactions (infectious mononucleosis), and toxic states (chronic hepatitis) as well as in certain other diseases (chronic lymphatic leukemia). In these instances the "reticular lymphocytes," because of the marked proliferation in the organs are crowded into the peripheral blood. (The "blast" form is seen in peripheral blood smears in leukemic reticulo-endotheliosis—otherwise it is seen in lymphoid tissue only.

2. The second type of heteroplastic regeneration is that associated with acute and subacute lymphatic leukemias. In this second type a stem cell is interposed. Downey discriminates between acute lymphatic leukemia with high white cell count and that with low white cell count. In lymphatic leukemia with high white cell the undifferentiated reticular cell gives rise to a stem cell which proliferates and transforms into immature forms which upon further proliferation and transformation become mature lymphoctyes. But because the hematopoietic organs become packed with these immature forms a great many of them are crowded into the circulating blood. These cells differ from the lymphocytes normal to the lymphatic organs and bone marrow of human blood.

These immature cells closely approach the myeloblast for nuclear structure and are not identical to the "reticular lymphocytes" which bear a resemblance to both the reticulum and to the lymphocytes of the blood stream and are sometimes referred to also as "immature lymphocytes." It is important to be able to recognize the various types of immature lymphocytes. The size, shape, and ratio of nuclear diameter to cell diameter is valueless. The most valuable critera are the disposition of the chromatin and parachromatin in the nucleus, and the presence or absence of nucleoli.

In acute lymphatic leukemia with a low white cell count and little evidence of hyperplasia of the lymph nodes, the stem cells are derived by dedifferentiation of lymphocytes. In health, the stem cell (myeloblast or lymphoblast) is not seen in the lymph node.

Lymphocytic potentialities. 1. Although the normal lymphocyte in the blood stream is a relatively inactive cell it may migrate into loose connective tissue, survive, and give rise to epitheloid cell, macrophages, microphages (polyblasts or histiocytes), tissue mast cell, plasma cell, or pass on into a fibroblast as said before. Sometimes the lymphocytes enlarge to such an extent that they become identical with the histiocyte budded directly from the reticulum. 2. Under experimental conditions and in pathological myeloid metaplasia lymphocytes play extraordinary roles.

a. In tissue culture of lymph nodes, Maximow demonstrated that the reticulum budded off large lymphocytes directly, these differentiating into granulocytes by first dedifferentiating into stem cells. Small lymphocytes were seen to develop into small myeloblasts and these into granulocytes.
b. In instances where the physiological stimulation is due to toxic substances used experimentally or due to processes associated with myelogenous leukemias and leukemoid reactions, lymphocytes in lymphoid tissue differentiated into macrophages, monocytes and granulocytes. This was accomplished by (1) All lymphocytes keeping lymphocytic characteristics for a time then becoming granulocytes, or (2) Lymphocytes dedifferentiating into stem cells, then myelocytes and finally mature granulocytes. This latter is a variety of reversed heteroplastic regeneration, i.e., a differentiation of lymphoid tissue into myeloid tissue (and not myeloid tissue of extra lymphatic origin). (3) Lymphocytes are valuable stores of protein. Erick and Harris showed that the adrenotropic hormone of the pituitary gland causes an increase of adrenocortical hormones affecting lymphoid tissue causing a lymphopenia and atrophy of the thymus. Dougherty and White showed by injecting adrenocortical extracts into adrenalectomized mice and normal rabbits that marked changes occurred in the lymphoid tissue as early as one hour after injecting. It appears that the corticosterone extracts are influenced by the adrenotropic hormone and the mechanism is responsible for release of gamma globulins (immune bodies) by the dissolution of lymphocytes.

Destruction of Lymphocytes. Lymphocytes may migrate from the blood stream into other tissue, survive and become transformed into any other type of cell. Most of the tissue lymphocytes produced in tonsillar and other lymph follicles and in diffuse adenoid tissue of the mucous membrane *especially Peyer's patches,* emigrate from mucous surfaces and are lost in the stool or by dissolution due to corticosterone hormones.

Alterations may occur in leukocytes other than the cytomorphic normal alterations concomitant with the process of differentiation. Cells undergoing such changes have "oid" affixed to the name. These changes may be due to aging, or cellular adaptations from which the cell recovers when conditions are altered. The description of a leukocytoid lymphocytes is this: The diameter is 10 mu-15 mu. The *nucleus* remains ordinary size but becomes polymorphic and eccentric, the pattern consisting of coarser, and heavier than normal *chromatin,* with more distinct *parachromatin.* Generally there are no *nucleoli.* The *cytoplasm* may increase in quantity with an increase or decrease in basophilia—a mottling due to yellowish hyaloplasm intermixed with bluish spongioplasm. Some-

times it is vacuolated. The *granules* remain azurophilic but become larger and darker. *Auer bodies* may be seen in some.

Such cells are seen in infectious mononucleosis and may be seen also in lymphatic leukemias and in agranulocytosis even to the extent of 100 per cent, but seldom in normal blood. Toxic lymphocytes are such as may demonstrate pyknotic nuclei, or an irregularity in structure, with foamy or more basophilic cytoplasm and vacuolization; and heavier granulation. Toxic lymphocytes are seen in toxic anemias. Sometimes the plasma cells is the product of toxicity in lymphocytes.

Lymphocytosis. Under three conditions are the lymphocytes sufficiently altered as to number and nature that without clinical data one cannot recognize the blood picture. These conditions are: a. Lymphocytosis, b. Leukemia, and c. Leukemoid reactions, e.g. infectious mononucleosis. While a white cell count that exceeds the maximum normal white cell count (normal range 7,000-10,000) is called a leukocytosis, when the question pertains to one cell in the differential, the condition is called an absolute leukocytosis rather than relative. A relative leukocytosis means that on the basis of a hundred white cells the ratio of the cell in question, and in this instance, the lymphocyte, is elevated, but the absolute number is not necessarily increased.

An absolute lymphocytosis may be seen in mumps, pertussis, measles, brucelloses, chronic and acute lymphatic leukemias, convalescent stage of tuberculosis, Hodgkin's disease, rickets, congenital syphilis, typhoid fever and infectious mononucleosis, infectious lymphocytosis and diseases of the thyroid and adrenal glands.

In pertussis there is an example of particularly children's response to infection—the increase being a lymphocytosis with a white cell count of 20,000 to 100,000 with 80-90 per cent lymphocytes in three sizes with occasionally an immature lymphocyte, plasma cell and leukocytoid lymphocyte. The majority of the cells are mature otherwise or at least possess none of the immaturity associated with acute lymphatic leukemia. Should there be myeloblast-like cells present they are in the peripheral blood for two reasons: They are a part of the normal cycle of lymphocytic development and second, children have a labile and unstable hematopoietic system and pertussis is a childhood disease.

Relative lymphocytosis may be found in almost all the conditions associated with a leukopenia, especially of the enteric fever group.

A lymphopenia may be seen in yellow fever, agranulocytosis, prolonged exposure to X-rays, typhus fever, and in children particularly at the height of pyogenic infections, the lymphocytes may be relatively low though the white cell count may reach 100,000.

Infectious lymphocytosis: This disease was first reported by Smith in 1941, and is a communicable condition seen in infants

and young adults and spreading to family or wider epidemics. It persists 2½-7 weeks and disappears spontaneously. Clinically the symptoms are those of a mild upper respiratory infection with abdominal pain, vomiting as in appendicitis with no adenopathy or splenomegaly. The blood picture shows a white cell count of 40,000-125,000 with 50-90 per cent of the cells lymphocytes. During convalescence there may be an eosinophilia of 14 per cent. The bone marrow is like that of lymphatic leukemia with 40-50 per cent lymphatic tissue.

One of the most frequently met leukemoid reactions involving lymphocytes is the lymphoid type known as infectious mononucleosis (glandular fever or acute benign lymphadenosis). It mimics acute lymphatic leukemia.

By definition infectious mononucleosis is a clinical entity seen in children, and young adults, characterized by a sudden onset, a few weeks' duration, usually three, with comparatively rapid recovery. Clinically there are cervical adenopathy and sometimes splenomegaly and hepatomegaly. In severe cases there are stomatitis and hemorrhages—purpuric tendencies. (These symptoms are not necessarily specific to infections mononucleosis.) There is a severe sore throat usually, and a fever reaching 103°F., irregular, intermittent and falling by lysis. Other symptoms may refer to the abdomen, skin (rash like measles or syphilis) and cerebral symptoms with meningeal irritation and paralysis. The blood picture is usually less variable than the clinical. At first there may be a low white count but early it becomes a leukocytosis of 10,000-14,000 rarely higher with a relative—59-92 per cent—and always absolute lymphocytosis. The lymphocytosis persists more or less into convalescence when the lymphocytosis is without atypical cells. There are in most cases large lymphocytes, some small and medium sizes besides atypical lymphocytes which Downey designates as Type I, Type II, and Type III. The Type I cell is the one described under leukocytoid lymphocyte title immediately above. Type III is the reticular lymphocyte in the "Lymphoid Cells Compared" table given earlier. Type II lymphocyte is a plasma-like cell according to Downey. It is relatively large in diameter; *nucleus* is round, with sharper demarcation between chromatin and parachromatin than in normal lymphocyte; *nuclear membrane* is distinct but *no* nucleoli; the *cytoplasm* is abundant and flaring with peripheral basophilia. It may be vacuolated—but in general it is smoother than Type I cytoplasm. The granules may be increased in size, shape, number, and depth of stain or there may be none at all.

Not every case of infectious mononucleosis has the same variety of lymphocytic cells, but usually an increased number of peculair young lymphocytes—not the "blast" type. Sometimes there is a

decrease in the number of platelets; and usually moderate or no anemia.

Its diagnosis is difficult but is made best from a smear, case history, absence of bacterial etiology, and degree of involvement of bone marrow as a rule. There is a positive heterophile antibody test in 90 per cent of the cases, a falsely positive Wassermann, an increased total white count without nucleated red blood cells and usually normal number of platelets.

It may be confused with 1. Acute lymphatic leukemia but the latter usually has a higher white count and decreased number of platelets in spite of sore throat, high fever, cervical adenopathy and 80-90 per cent lymphoblasts. 2. Serum sickness (ruled out because of lesser positive titre in heterophile antibody test). 3. Chronic lymphatic leukemia—not acute in onset; a monotony of lymphocytes as to size; white cell count much higher reaching 1,000,000; and the age of the patient—usually too old. 4. Undulant fever. 5 Agranulocytosis—low white cell count—here a relative lymphocytosis with all toxic leukocytoid lymphocytes. 6. Pertussis—higher white cell count, lymphocytes are all sizes and shapes. 7. Leukemic reticulo-endotheliosis—numerous reticular lymphocytes and numerous hematopoietic reticular cells with practically no leukocytoid lymphocytes. 8. German measles—9000 white cell count with leukocytoid lymphocytes and plasma cells increased above 4 per cent. 9. Typhoid fever—10 per cent monocytes. 10. Infectious lymphocytosis. 11. Infectious hepatitis with presence of plasma cells and relative lymphocytosis. The conditions of the liver may aid in establishing this diagnosis.

HEMOPOIETIC HYPERPLASIA*

	Subactive Lymphatic Leukemia	Infectious Mononucleosis
Reticulum	Hyperplasia more uniform	Hyperplasia patty, spotty
Reticular cells	None in peripheral blood	Present and resemble reticulum
Recovery as evident from biopsy	Hyperplasia gone but few abnormal cells in peripheral blood	Hyperplasia gone in nodes with no abnormal cells in blood

* Infectious mononucleosis and leukemia have one common factor—the involvement of the reticulo—endothelia system.

Thus, perhaps excluding chronic lymphatic leukemia and mature lymphocytes, lymphocytoses and lymphocytes form an interesting and sometimes puzzling picture.

A NEW SEROLOGICAL PIPETTE*

**DANE W. MOORE JR., B.S., M.T. (ASCP)

This describes a new serological pipette especially designed for use in the VDRL and similar slide flocculation tests but also useful in other serologic tests.

The figure shows the dimensions and calibration of the pipette. The length is 242 mm., the body of the pipette is 7 mm. in diameter, the tip begins 30 mm. from the end and tapers to 3 mm. The bore measures 2 mm. in diameter in the body of the pipette and 1 mm. at the end of the tip. Each graduation encircles the pipette.

Table I

NAME OF TEST	Amount Serum Used
Eagle	0.4 ml.
Hinton	0.5 ml.
Kahn	0.15 ml.
Kilne	0.05 ml.
Kolmer	0.2 ml.
Mazzini	0.05 ml.
Rein-Bossak	0.05 ml.
VDRL	0.05 ml.

Table I shows the amounts of serum used in each of the standard serological tests. From this table it is seen that none of the tests employs more than 0.5 ml. of serum and all of them employ either 0.05 ml. or multiples of this so that the capacity and the graduations of the pipette would appear ideal.

The greatest advantages of this pipette accrue from its shortness. The advantages over a longer pipette are: it is easier to pipette material on to a ringed slide or a concavity slide because the tip is easier to control; it is not so tiresome to use because the pipetting arm is not held so high; there is less danger of knocking over a rack of test tubes containing the pipettes; less space is taken in the refrigerator with the rack; a smaller jar accommodates them after use; and they are easier to clean.

Other advantages are that it is easier to read because the graduations are simpler and when the pipette is picked up it does not have to be turned to see the graduation figures. In the VDRL test the serum can be drawn to any mark and let down to the next mark. This saves time and it is easier to do than with any other type of pipette.

Although the pipette is designed especially for pipetting serum it can be used for preparation of the working antigen in most of the tests. The pipette is constructed so that the tip is long and small enough to fit into the glass ampuls in which some of the commercially prepared antigens are supplied.

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* This pipette is available through Scientific Products Division, American Hospital Supply Corp., 1210 Leon Place, Evanston, Ill.

** From the laboratory of the Myers Clinic Hospital, Philippi, West Virginia.

A TECHNOLOGIST'S TRIALS AND TRIBULATIONS

Though the "noble nurse" gets all the glory—
Technologists, too, have their story.
The "special" answers to one "Doc";
The "lab. girl" has to please a flock.
Here's a guy or gal who fares far worse
Than any stiff-starched graduate nurse.
He looks at pus and works with gore,
With orders coming by the score;—
This one for a count on Kelly
Who has a bad pain in his belly,
A "coag-time" on Mr. Cropt
Whose bleeding nose they can't get stopped,—
A blood culture, now, on Betty Gates—
It seems her temperature fluctuates.
"Do this urine right away—
'Cause they might operate today."
A blood sugar, "stat," on Mrs. Stock,—
They think she's having insulin shock.
Mrs. Denny has an enlarged throat,
A B.M. rate to her we vote,
This kid has a running ear,
And Dr. Sam would like a smear.
Boils "break out" on little Maxine,
Culture one and make a vaccine.
A "typing" order for Mrs. Drew,
Get her a donor by half past two.
A Friedman test on Mrs. Wither,
At present she is all "a-dither"!
Bob Spratt coughs continuously— .
Examine his sputum for t.b.
Now, that case of bowel obstruction—
Do the chlorides show reduction?
Here's a bag full of house dirt,
Make an "extract" for Mrs. Wirt.
Please report—don't be a dud,
About calcium in this man's blood.
Stain this vaginal smear and see
If you can find any g.c.—
O all day long — item for item,
This goes on — ad infinitum!
But, here's a fact you must concede,—
Without us, our M.D.'s would be tree-d.

From the Journal of the Wisconsin Association of Medical Technologists. Mar.-Apr., 1954

THE GLUCOSE TOLERANCE TEST: AN AID IN THE DIAGNOSIS OF DIABETES MELLITUS*

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It was with a great deal of pleasure that I accepted the invitation to address you this afternoon. Being with you to discuss this phase of a sometime difficult problem, the diagnosis of diabetes mellitus, reiterates that the success of the physician in arriving at a conclusive diagnosis is so vitally linked with and depends in a large measure on you, the members of the American Society of Medical Technologists, in the performance of your tasks. With your aid, the treatment, the progress, and the many crises of diabetes mellitus are properly evaluated and cared for. So with this realization in mind, I have chosen for my subject—The Glucose Tolerance Test: An Aid in the Diagnosis of Diabetes Mellitus. Various aspects of this subject have been thoroughly covered by many publications in the medical literature so therefore it is not the purpose to make any new contribution, but rather to summarize, in so far as possible, the pertinent points relative to our discussion and consideration. Therefore, with an interest in the disorder of diabetes mellitus, I hereby acknowledge and express a deep appreciation to the investigators in this field.

The presentation of this review will be discussed in the light of the following topics.

(1) General consideration of the problem
(2) Indications and contraindications for the test
(3) The tests generally employed and pertinent features of each
(4) The Standard glucose tolerance test with a discussion of the technique and results
(5) The features of a normal and abnormal response
(6) Interpretation in general
(7) Discussion and conclusion

General Considerations: A test or group of tests cannot in reality be substituted for a well organized plan in the diagnosis of any disease, and one must remember that whenever a diagnosis is made on a single test or group of tests, eliminating the consideration of the clinical picture and other evidence for interpretation, it will result not only in a high percentage of error but untold hardship on the patient as well as a reflection on the medical profession. Thus, in making a diagnosis of diabetes mellitus, the following shoud be considered: (1) the patient's symptoms, (2) the family history of diabetes mellitus, (3) the patient's past history, (4) the findings on physical examination, and (5)

*Read before ASMT, Louisville, Ky., June 1953

the essential and necessary laboratory data. The evidence gained from the first four mentioned above arouse the suspicion of diabetes and should lead one to further investigate with the hope of confirming or disproving the suspicion or the tentative diagnosis of diabetes mellitus by the appropriate laboratory tests.

It is assumed for the purpose of this discussion that the individual has some essential feature as presented above and the suspicion that the individual has the disorder of diabetes mellitus. Further that an exhaustive survey has been made to confirm this suspicion by the use of many of the simple available tests, namely a fasting blood sugar and urinalysis, or a blood sugar and urinalysis following an ordinary or test mea. It is now assumed that in spite of these procedures one is unable to confirm the tentative diagnosis of diabetes which has been entertained. This now becomes a so-called borderline or doubtful case and in order to properly classify and make a conclusive diagnosis, the use of a valuable tool, namely the glucose tolerance test, may very well bring a proper solution to the problem.

In general the glucose tolerance test is indicated when the diagnosis of diabetes mellitus cannot clearly be made without its use. The procedure then as stated before is clearly indicated in individuals with a history of glycosuria, a family history of diabetes, evidence of transitory glycosuria and hyperglycemia as may occur in such as infections, pregnancy, hepatic disorders, hyperthyroidism, coronary thrombosis, starvation and glycosuria as occurs in renal glycosuria, thyrotoxicosis and nephrosis without the concomitant hyperglycemia and in whom the preliminary investigation has not been conclusive.

Before instituting this procedure great care shouldl be taken to see that the patient is in the best possible physical condition in order that the results obtained may be properly evaluated on factual results. These include the following considerations: A history and physical examination to determine if there are any pre-disposing factors which would result in an abnormal or diminished glucose tolerance curve. Therefore an infection, hepatic disturbance, frank diabetes, previous dietary restrictions, obvious dibilitating diseases requiring prolonged bedrest are definite contraindications. It must be kept in mind that every diabetic will show a high and usually prolonger blood sugar curve, but every case of a person presenting such a curve, is not a diabetic.

In preparation of the investigation it is well to point out some salient features again which are commonly overlooked. The patient should be in a fasting state, not having partaken of food since the previous evening, the average duration being approximately 12 hours. The temperature should be taken at the beginning of and completion of the test in order to rule out any obvi-

ous infection. Factors which may influence the test, such as extreme nervous tension, smoking before the test, menstruation, some drugs, should cause a postponement of the test. The most important consideration is that the patient has had an adequate diet for a period of 3 to 5 days previously and such diet should consist of a daily carbohydrate intake of 200 grams or more. In case of a suspected situation that the individual does not have diabetes but has been taking insulin because of an erroneous diagnosis, the insulin should be discontinued for a period of at least 3 to 5 days before the test.

For investigation there are for general purposes three types of glucose tolerance tests. The essential features of each will be outined, but for the sake of brevity (and must admit preference) the so-called Standard (one dose) Oral Glucose Tolerance test will be used for the purpose of this discussion.

(1) *The one hour-two dose dextrose tolerance test* as introduced by Exton and Rose and based on the principles established by Hammon-Hirschmann. Essentially the patient is given 50 grams of glucose after the fasting blood sugar is drawn and a urine obtained. At the end of one-half hour a second blood sugar and urine is taken and another 50 grams of glucose are given. When another half-hour has passed a third blood sugar and urine is obtained. This test has its proponents but the criticisms in general are: (a) high incidence of inconclusiveness regarding the diagnosis in the borderline cases; (b) the test does not actually give an accurate estimation of the subject's ability to dispose of 100 grams of glucose, since by the end of the hour much of it will remain unabsorbed; (c) the test may depend entirely on the variable rate of absorption and not necessarily the rate of utilization; and (d) only the height of the blood sugar is expressed.

(2) *The intravenous glucose tolerance test.* In this a given amount of glucose is injected intravenously either in a specified time or continuously and blood sugar samples taken at varying intervals of time and duration depending upon the investigator. This test is primarily recommended in cases of abnormality of absorption of glucose from the intestine such as may occur in a gastroenterostomy and in research, but as a general rule it is not.

(3) *The Standard Glucose Tolerance Test.* This is probably the choice test of most workers particularly in the clinical field. An advantage necessarily lies in the fact that it presents the height and duration of the glucose curve. The following technique is empoyed: The patient reports in the morning in a fasting state, not having had any food since the previous evening, approximately 12 hours, and having had an adequate diet of approximately 200 to 300 grams of carbohydrate for 3 to 5 days previously. The bladder is emptied and a sample of blood (venous-5cc) for a sugar determination is taken. Then 100 grams of glucose dis-

solved in 250 to 300 cc. of water flavored with the juice of a lemon is given by mouth. This should be properly chilled. (In children, approximately one gram/lb. body weight). Specimens of blood and urine for sugar determination are secured in the fasting state, one-half hour, one hour, two and three hours following the administration of the glucose.

Blood may be taken either from a vein, finger, or ear. If capillary blood is used it must be borne in mind that the values are higher. The exact figures are variable from 20 to 50 mg. per cent, more or less depending on the test or its modification. The value obtained from venous and capillary blood sugar determinations taken in the fasting state for all practical purposes are the same. It is only after the test dose that the higher values are obtained as regards the capillary blood.

Though at present many modifications of the Folin-Wu method of blood sugar determination exist, we will take the time only to mention that in the last few years the trend has been away from the use of the Folin-Wu to the use of the true blood sugars as determined by the Somogyi modification, as well as many other modifications. The blood sugar value as determined by Folin-Wu procedure includes the non-glucose, non-fermentable reducing materials as sugar, i.e., glutathione in particular. The values for these materials have been found to vary from 10 to 80 mg. per cent.

In general, whatever method and modifications are used as stated above, and being well aware of the marked importance of obtaining the true blood sugar values, and because the entire procedure has not been clearly standardized as to either the methods or values, it is my feeling that for the present it should rest on one's experience with a test and his ability to interpret it in this light.

At present the important consideration is whether or not the curve obtained by the previous efforts is representative of a normal or abnormal response. The normal curve reveals a fasting value below 120 mg. per cent. Following the administration of the glucose the blood sugar rises rapidly reaching a maximum in thirty to sixty minutes. Usually the value does not exceed 140 mg. per cent. Within two hours the blood sugar should have fallen to 120 mg. per cent or below. This represents that the individual is adequately able to handle glucose.

By a diminished or abnormal glucose tolerance response is meant the inability of the individual to handle ingested glucose as efficiently as a normal individual. This is represented as being decreased tissue utilization expressed as the diminished ability of the extrahepatic tissue to form glycogen and to utilize glucose with a resultant excess of glucose remaining in the blood. Such a diminished glucose tolerance, sometimes called the ex-

aggerated response, will have the following characteristics:

(1) The fasting blood sugar may be within the accepted normal range values or above (70 to 110 mg. per cent). As a general rule, the milder the diabetes, the less likelihood of a fasting hyperglycemia. Above 130 mg. per cent is considered diagnostic; above 120 mg. per cent as suspicious.

(2) A gradual rise to an excessively high value—160 mg. per cent and generally above 170 mg. per cent.

(3) Maximum concentration is reached after a variable period, usually in approximately one hour.

(4) A delayed return to the post-absorptive (fasting) level, usually not in less time than two hours.

(5) The specimens of urine contain sugar.

In general if the results obtained meet the requirements as have been elucidated before, confirming the tentative diagnosis which was entertained because of the clinical picture, the diagnosis is readily made. Yet there must be mentioned several points of divergence in regards to the interpretation of the curves obtained. This will serve to emphasize that though the tolerance tests are widely used, the interpretations vary greatly.

The various investigators may interpret the sugar tolerance curves according to the following:

(1) The peak of the blood sugar—If the Folin-Wu method is used a venous blood sugar value of 170 mg. per cent or above one hour after the administration of glucose with corresponding sugar in the urine specimen is considered confirmation of diabetes.

(2) The rate of blood sugar fall, the value being obtained at the end of two hours after administration of glucose using the method above. A two hour value above 130 mg. per cent is indicative of diabetes.

(3) A combination of the two above, that is both the peak and rate of fall. This latter method perhaps is the safest.

In the general sense, with any procedure, there will exist the borderline case in which the interpretation is questionable; therefore the test should be repeated at a later date, but not sooner than 10 to 14 days. It should be borne in mind that if the clinical picture is such that it continues to strongly suggest diabetes the glucose tolerance test may very well be repeated at intervals of three to six months. Certainly a single negative test should not exclude the possibility of making a diagnosis at some later date when the disorder may be more pronounced.

In conclusion the glucose tolerance test when carefully done, under controlled conditions and interpreted in light of specific standards and always correlated with the clinical picture, will give results which will prove to be a valuable aid in the diagnosis of diabetes mellitus.

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TWO KINDS

There are two kinds of people on earth today
Just two kinds of people—no more, I say,
Not the good and the bad, for 'tis well understood
The good are half bad and the bad are half good;
Not the rich and the poor, for to count a man's wealth
You must know the state of his conscience and health;
No, the two kinds of people on earth that I mean
Are the people who lift and the people who lean.
Wherever you go, you will find the world's masses
Are divided up in just these two classes.
And, oddly enough, you will find, too, I ween,
There is only one lifter to twenty who lean.

—*Selected.*

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AN INEXPENSIVE HEMATOCRIT CENTRIFUGE

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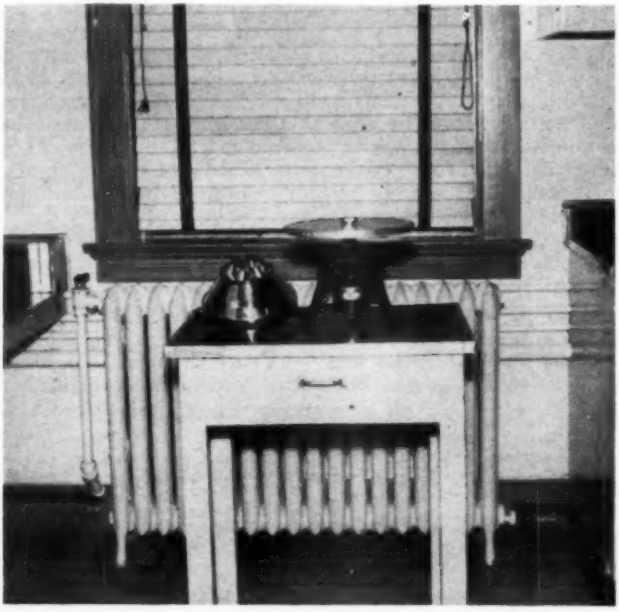
The hematocrit, if properly done, is considered to be one of the most accurate and simple methods for determining the packed red blood cell volume and other hematologic data. A technique frequently employed requires a Wintrobe tube filled to the 10 mark with oxalated venous blood and then centrifuging the tube in a horizontal position at a speed between 3000 to 4000 rpm until constant packing is attained. The time required to achieve this constant packing is usually 30 to 40 minutes, preferably without interruption. This time interval presented a problem in our laboratory in that it tied up a large floor model centrifuge for considerable periods of time and prevented the use of the centrifuge for other purposes.



In order to circumvent this problem and still perform accurate hematocrit determinations, we devised a centrifuge which was comparatively inexpensive and has proven to be equal in reliability to the large centrifuge in the determination of the hematocrit.

We used a "Servall"[1] table model angle head centrifuge type A. The angle head can be removed by simply lifting it from the drive shaft which extends upward from the motor. An International[2] eight-place centrifuge head, especially designed for hematocrit determinations using the Wintrobe tube, was fitted to the drive shaft by having a metal sleeve of the proper size welded into the center opening of the International centrifuge head. The metal sleeve, which can be fitted by any competent machinist, is a hard steel alloy measuring 34mm in length, 10mm inside bore diameter and 13mm outside bore diameter. The hematocrit head is placed on the drive shaft and is held firmly in place by tightening the set screw against the drive shaft. The properly filled Wintrobe tubes are then placed in the grooves, the lid placed on the head and tightened and the centrifuge is turned on to the proper speed. The speed is controlled by a rheostat that is part of the original "Servall" centrifuge equipment. The photograph, Figure 1, shows the centrifuge assembled and ready for use. When the hematocrit determinations have been completed, the centrifuge may be used for other purposes by replacing the angle head on the drive shaft. Changing heads on this centrifuge can be done in a few seconds and imposes no additional wear on the motor or drive shaft. The "Servall" table model centrifuge is well constructed and when placed on a solid table top will spin at the required speed with a minimum of noise and vibration.

This apparatus has been in use in our laboratory for about two years and has proven to be entirely satisfactory in that the hematocrit readings compare favorably with those obtained by using the floor model centrifuge. We feel that we have effectively removed a bottle neck in our laboratory and at the same time have "two" efficient centrifuges for the price of one plus the small additional price of the International hematocrit head.

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2. International Equipment Co. Number 289. Can be purchased from A. S. Aloe Co., St. Louis, Missouri.

DEVELOPMENTS IN THE FIELD OF VENOMS, ANTIVENOMS AND SNAKEBITE TREATMENT

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From earliest history snakes have been the subject of voluminous writings. It is doubtful whether any other animal has provided for so many stories involving so little truth. Casual observations, limited knowledge and unlimited imagination have resulted in many fantastic accounts. Some of these tales have been repeated so often that it is now difficult to convince intelligent persons that many of the stories are completely without foundation. It is perhaps logical for snakes to create a variety of interests—mingled reactions of fear, horror, respect and curiosity—since they are unique in many respects. Although without limbs, snakes can travel rapidly on land, climb trees and swim with ease. Some varieties produce venoms which are among the most toxic substances known. The complex nature of these venoms is indicated by the fact that comparatively little is known concerning their exact composition in spite of the efforts of many research workers. "Bibliography of Animal Venoms"[1] lists over 4100 references covering the period from 1875 through 1946. Many of the reports of early studies are of little scientific value as details and controls are lacking.

Accurate records of poisonous snake bites are difficult to obtain. Estimates[2] indicate that there are two to three thousand cases in the United States annually. From three to five per cent of these occur in Florida.[3] Twenty thousand deaths per year from snake bites have been reported in India.

With the progress in scientific fields came the development of antivenoms for treating victims of snake bite. In view of the variety of poisonous snakes, which are widely distributed over the world, various antivenoms designed to meet the needs of geographic locations are produced in several countries.

In the Bangkok area of Thailand, with an estimated population of one million, there are about 1300 poisonous snake bites per year. However, due to the production of antivenom which is effective against the venoms of the snakes of that area and improved facilities for treatment, the death rate is exceedingly low.[4]

The poisonous snakes which are native to the United States include the coral, the cottonmouth moccasin, the copperhead and at least 26 kinds of rattlesnakes. Two of these rattlers, *Crotalus adamanteus* (Eastern or Florida Diamondback) and *Crotalus atrox*

(Western or Texas Diamondback) are among the world's most dangerous snakes.

The number of poisonous snakes and the range of varieties handled by scientific workers in this country are greater than usually is realized. Ross Allen's Reptile Institute at Silver Springs, Florida, milked 73,000 snakes (27 varieties) and processed the venom for the use of our Armed Forces during World War II. This institute is recognized internationally for its ability to furnish almost any animal venom needed in scientific investigations.

Numerous concoctions and techniques have been proposed for snakebite treatment. Many of these proposals fall into the category of witchcraft. The literature reveals little information on research of treatment or reliable details of case histories. Andrews and Pollard[3] were the first to report any appreciable data on hospitalized cases in Florida. The most recent cases in their report involved first-aid treatment, use of ice packs, Antivenin, tetanus-gas gangrene antitoxin, antibiotics, whole blood transfusions, glucose-saline infusions and Benadryl. Watt and Pollard[5] recently reported successful treatment of a very serious Florida Diamondback bite case. In this case the patient was administered five ampoules of Antivenin on admission to the hospital. Whole blood transfusions were given in the early stages of treatment, and ice packs were used continuously for eight days. No necrosis of tissue resulted. It appears that the use of ice packs as an integral part of treatment warrants continuation. In numerous cases in which ice packs were employed little or no necrosis of tissue has resulted.

In two cases involving Florida Diamondback bites on hands the treatment was the same except that ice packs were not used in Case No. 1 (Figure 1), while they were used during the first few days in Case No. 2 (Figure 2).

The value of Antivenin, Nearctic Crotalidae (North American Antisnakebite Serum), formerly produced as a specific treatment of snake bite, has been repeatedly demonstrated. This serum had high titers against the venoms of rattlesnake, cottonmouth and copperhead, but its value against coral venom was very slight. After prolonged research Wyeth Laboratories have produced a new, improved Antivenin to supplant this earlier product. The new antiserum will afford better protection against the pit vipers of North America, as it exhibits higher titers against the venoms of these snakes. It will also afford protection against the venoms of Central and South American crotalids and will be of value for treatment of bites by tropical crotalids which are imported for venom to be produced for medicinal use.

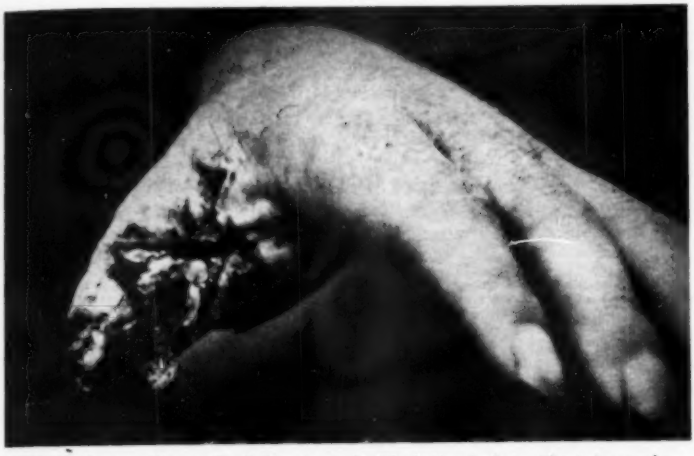

Fig. 1—Eastern Diamondback bite treated with Antivenin but without ice packs.

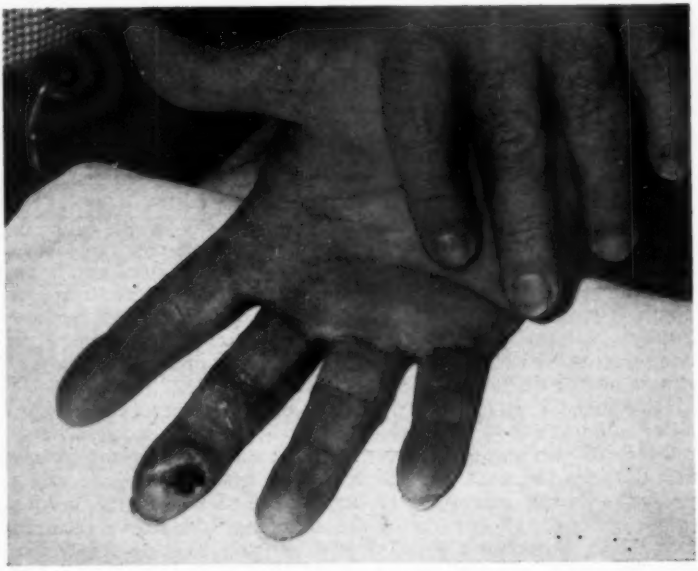

Fig. 2—Eastern Diamondback bite treated with Antivenin and with ice packs.

Wyeth Laboratories' new refined Antivenin (Polyvalent North and South American Antisnakebite Serum) is prepared by injecting a mixture of *Crotalus atrox, Crotalus terrificus, Crotalus adamanteus* and *Bothrops atrox* venoms into horses. Blood is withdrawn from the horses when antibodies are at a high level. Antivenin is processed from the withdrawn blood.

Table 1 gives data concerning LD_{50} values of venoms and titers of the new Antivenin (Crotalidae) Polyvalent (North and South American Antisnakebite Serum) based upon recent tests. All titrations were done on 18-gram mice, intravenously.

TABLE 1

Data Concerning LD_{50} Values of Venoms and Titers of Antivenin (Crotalidae) Polyvalent. (North and South American Antisnakebite Serum) Based Upon Recent Tests. (All titrations done in 18 gram mice, intravenously)

VENOM	LD_{50} per mg. of venom	mg. of venom per LD_{50}	Antivenin Neutralization Tests	
			mg. of venom neutralized by 1cc Antivenin	LD_{50} neutralized by 1cc Antivenin
Crotalus atrox	18	0.056	1.15	20.5
Crotalus adamanteus	36	.028	1.75	62.5
Crotalus horridus	18	.056	1.6	28.6
Crotalus viridis	50	.020	1.1	55.0
Crotalus oreganus	22	.045	1.5	33.3
Crotalus ruber	14	.071	2.7	38.0
Crotalus basiliscus	5	.200	2.3	11.5
Crotalus terrificus	440	.0023	0.42	184.7
Agkistrodon mokasen	8	.125	2.8	22.4
Agkistrodon bilineatus	34	.029	1.4	48.3
Agkistrodon piscivorus	16	.063	2.55	40.5
Bothrops atrox (Fer de lance)	54	.018	2.1	117.4
Bothrops neuwiedii	44	.023	2.05	89.0
Bothrops jararaca	44	.023	1.7	74.8
Bothrops jararacussu	3.2	.313	2.25	7.2
Bothrops alternatus	50	.020	0.9	45.0
Lachesis muta	38	.026	1.5	57.7

Since many of our most useful drugs are in reality poisons, it is not surprising that snake venoms have attracted the attention of some research workers who were seeking new drugs for combatting diseases. Although the chemical structures are not known, it has been established that various venoms contain components which are hemolytic, neurotoxic, proteolytic, hemorrhagic and antihemorrhagic in nature.

Obviously, it is beyond the scope of this paper even to consider a complete review of literature involving such a large number of publications. Interest in the field is reflected by the fact that venoms have been or are being studied in regard to use as drugs in the treatment of intractable pain, osteitis, herpes, tabes, epilepsia, chorea, neurasthenia, hysteria, blackwater fever, thrombosis, bronchial asthma, angina pectoris, nephrosis, purpura, post-operative hemorrhage, uterine hemorrhage, hemophilia, cancer and polio.

The use of cobra venom in clinical therapeutics has been developed by both foreign and American investigators. Charles Taguet and associates[1] of France and D. I. Macht and collaborators[1] of this country have published numerous reports. Varying degrees of success have been reported in the use of cobra venom for the relief of pain in cancer. At present the use of cobra venom for intractable pain is probably more general in foreign countries than in the United States.

The venoms of several snakes, notably *Russell's viper, Bothrops atrox* and *Agkistrodon piscivorus,* have been reported as valuable in controlling hemorrhagic conditions. Samuel M. Peck and coworkers[1] have published numerous articles concerning the use of moccasin and *Bothrops atrox* venoms. *Russell's viper* venom is in general use in Thailand for the treatment of hemorrhagic cases.[4] B. Jansky[6] reported a study of the action of *Bothrops atrox* venom on fibrinogen.

In recent years considerable interest has developed in enzymatic studies of venoms. L. A. Heppel and R. J. Hilmoe[7] reported some of their studies in this field in an article which carried 19 literature references.

Since 1946 over 200 research reports dealing with various medical aspects of venoms have appeared in the literature. It is interesting to note that approximately three-quarters of these publications appeared in foreign journals.

The field involving venoms and antivenoms offers a compelling challenge to researchers in medical sciences. Additional hemological, pathological, serological and clinical chemical studies should be encouraged. Detailed medical case histories would be beneficial to those who are shouldered with the responsibility of treating snake bite cases. Certainly much of the progress which is made in treatment of venomous bites and in therapeutic use of venoms will be dependent upon "control data" furnished by the clinical laboratory.

The author is indebted to Dr. B. Scott Fritz and Mrs. Eleanor Buckley, Wyeth Laboratory Incorporated, for Antivenin titers.

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THE DETERMINATION OF ACID AND ALKALINE PHOSPHATASE USING p-NITROPHENYL PHOSPHATE AS SUBSTRATE*

ANNE J. SOMMER, B.S., MT(ASCP)

* Published in the Saint Louis University Medical Bulletin, Vol. 4, No. 9, pp. 165-9, November, 1952, and read before ASMT, Louisville, Ky., June, 1953.

The investigations of Kay,[1] Woodard, Twombly, and Coley,[2] and Roberts,[3] showed that the serum alkaline phosphatase is elevated in liver disease or when there is increased osteoblastic activity. Gutman and Gutman,[4] Barringer and Woodard,[5] and Huggins and Hodges[6,7] showed a relationship between carcinoma of the prostate and an elevation of serum acid phosphatase. These studies have produced a demand for rapid, accurate methods for the estimation of alkaline and acid phosphatases.

The estimation of serum phosphatase depends on determining the rate of hydrolysis of a monophosphoric acid ester in a suitable buffer. Sodium β-glycerophosphate and disodium phenyl phosphate are the two substances most commonly used. Determination of the amount of liberated phosphate and phenol respectively are used to indicate phosphatase activity.[8,9]

In 1946, Bessey, Lowry, and Brock[10] devised a method for the determination of serum alkaline phosphatase using p-nitrophenyl phosphate as the substrate with the liberated p-nitrophenol being determined directly in a photoelectric colorimeter. This method requires only minute amounts of serum and eliminates the precipitation of protein, making possible the analyses of 50 to 100 samples in 2 hours. The method was modified by Andersch and Szczypinski[11] so that serum acid phosphatase could be determined. The speed and extreme simplicity of this test recommend it as the best available method for the determination of the phosphatases.

Principle of the Test

The compound, p-nitrophenyl phosphate, is colorless, but upon hydrolysis of the phosphate group, the yellow salt of p-nitrophenol is liberated (absorption maximum, 400 mu). Thus, the substrate is itself an indicator of the amount of splitting and hence is a measure of phosphatase activity, as indicated by the following reaction:

$$\underset{\text{colorless in acid and alkali}}{\text{p-nitrophenyl phosphate}} + H_2O \xrightarrow[38^\circ\ C.]{\text{Phosphatase}} \underset{\text{colorless in acid yellow in alkali}}{\text{p-nitrophenol}} + H_3PO_4$$

The serum is incubated with the buffered substrate; the reaction is stopped by the addition of alkali; and, without further treatment, the amount of color developed is measured.

Early investigators[10] noted that the Eastman product (p-nitrophenyl phosphate) contained about 50% inert material. In addition, when preparing a solution, extractions with butyl alcohol and ether were required to remove the free yellow p-nitrophenol. In August, 1951, the Sigma Chemical Co.* prepared a white crystalline p-nitrophenyl phosphate which is stable when kept cold and whose solutions do not require extractions.

Preparation of Reagents

Alkaline Phosphatase

1. Alkaline Buffer Solution:

Aminoacetic acid (Glycine)	7.5 gm.	(0.1 mol)
$MgCl_2$	0.095 gm.	(0.001 mol)
Water	750.0 mo.	
1N NaOH	85.0 ml.	(0.085 mol)

Dilute to 1 liter with water.

2. Stock Substrate Solution:

p-Nitrophenyl phosphate, disodium	0.4 gm.
Water	100.0 ml.

3. Alkaline Buffered Substrate:

Mix equal parts of solutions 1 and 2. If necessary, adjust the pH to 10.3 or 10.4 with concentrated HCl or NaOH, using the Beckman or other pH meter.

Store the buffered substrate in the refrigerator or dispense into small test tubes and freeze to maintain stability. Decomposition of the buffered substrate may be detected by mixing 2 ml. with 10 ml. of 0.02N NaOH. The optical density should not exceed 0.15 in a 19 x 150 mm. cuvette when measured in the Coleman Jr. spectrophotometer against a water reference at 410 mu.

4. 0.02N NaOH
5. Concentrated HCl

Acid Phosphatase

1. Acid Buffer Solution:

Citric Acid (1 H_2O)	18.907 gm.	(0.09 mol)
1N NaOH	180.0 ml.	(0.18 mol)
0.1N HCl	100.0 ml.	(0.01 mol)

Dilute to 1 liter with water.

2. Stock Substrate Solution:

p-Nitrophenyl phosphate, disodium	0.4 gm.
Water	100.0 ml.

* Bulletin 104, Sigma Chemical Co., 4648 Easton Ave., St. Louis, Mo.

3. Acid Buffered Substrate:
   Mix equal parts of solutions 1 and 2. If necessary, adjust the pH to 4.8 or 4.9 with concentrated HCl or NaOH, using the Beckman or other pH meter.
   Store the buffered substrate in the refrigerator or dispense into small test tubes and freeze to maintain stability. Decomposition may be detected by mixing 1 ml. with 5 ml. of 0.1N NaOH. The optical density should not exceed 0.1 in the spectrophotometer against a water reference at 410 mu.
4. 0.1N NaOH.

Procedures

In the following procedures a Coleman Model 6 Spectrophotometer was used. However, any spectrophotometer or any photelometer which is fitted with a light filter which transmits light with a sharp peak in the range of 400 to 420 mu. can be used.

Alkaline Phosphatase

Alkaline Buffered Substrate 1 ml. into each of two 19x150 mm. spectrophotometer tubes

Place in 38° C. constant temperature water bath for five minutes.

Add:

Serum	0.1 ml. into Tube A
Water	0.1 ml. into Tube B (Reagent Blank)

Incubate in 38° C. water bath for exactly 30 minutes.

Add:

0.02N NaOH	10.0 ml. to each tube

Mix by inversion using rubber stoppers.

Adjust spectrophotometer to 0 (optical density) using reagent blank (Tube B) as reference at a wave length of 410 mu. Read the optical density of the serum sample (Tube A).

Add:

Conc. HCl	0.1 ml. (2 drops) to Tubes A and B

Mix. Repeat the determination as described above. This is the serum blank. Subtract the optical density of the serum blank from that of the serum sample. This is the *Corrected Optical Density*. Refer to calibration curve (see below) for alkaline phosphatase activity. When a value greater than 10 mM units is obtained, repeat using a smaller quantity of serum or a shorter incubation time.

Acid Phosphatase

Acid Buffered Substrate 1 ml. into each of two 19 x 150 mm. Spectrophotometer tubes

Place in 38° C. constant temperature water bath for five minutes.
Add:

Serum	0.1 ml. into Tube A
Water	0.1 ml. into Tube B

Incubate in 38° C. water bath for exactly 30 minutes.
Add:

0.1N NaOH	4.0 ml. to each tube

Mix. Adjust the spectrophotometer to 0 using reagent blank (Tube B) as reference at 410 mu. Read the optical density of the serum sample (Tube A). To correct the optical density for the amount of color contributed by the serum itself, add 5 ml. of 0.1N NaOH to 0.2 ml. of serum and determine the optical density of this mixture using 0.1N NaOH as reference. Subtract the optical density of the serum blank from that of the serum sample. This is the *Corrected Optical Density*. Refer to calibration curve (see below) for acid phosphatase activity.

Preparation of Calibration Curves for Serum Alkaline and Acid Phosphatases

Reagents:

1. p-Nitrophenol Standard Solution (10.0 millimols per liter).
   p-nitrophenol 0.1391 gm. (1.0 millimol)
   Water to 100.0 ml. in volumetric flask
2. "Working Standard" (0.05 millimols per liter).
   Standard Solution 5.0 ml.
   Water to 1000.0 ml. in volumetric flask
3. 0.2N NaOH.
4. 0.02N NaOH.

Into 19 x 150 mm. spectrophotometer tubes pipette the following solutions:*

ml. "Working Std."	ml. Water	ml. 0.2N NaOH	EQUIVALENT TO SERUM	
			Alkaline Phosphatase	Acid Phosphatase
1	9	1.1	1 mM unit	0.234 mM units
2	8	1.1	2 mM units	0.468 mM units
4	6	1.1	4 mM units	0.936 mM units
6	4	1.1	6 mM units	1.40 mM units
8	2	1.1	8 mM units	1.87 mM units
10	0	1.1	10 mM units	2.34 mM units

Read and record the optical density of each of the above mixtures at 410 mu. using 10 ml. of 0.02N NaOH as reference. A "millimol unit" is defined by Bessey, et al.[10] as "the phosphatase activity which will liberate 1 mM of p-nitrophenol per liter per hour." Since the term "mM unit" is non-specific and can be used to indicate the concentration of any pure substance, we would prefer to call this the "Bessey-Lowry unit" but have refrained

* Bulletin 104, Sigma Chemical Co.

from using this term to avoid confusion.

The optical densities are equivalent to serum alkaline phosphatase activities of 1, 2, 4, 6, 8, and 10 millimol units, respectively; and to serum acid phosphatase activities of 0.234, 0.468, 0.936, 1.40, 1.87, and 2.34 millimol units respectively. (If values of 0.2, 0.4, 0.8, 1.2, and 1.6 are preferred for calibration points for acid phosphatase, a separate set of dilutions may be prepared.)

As can be seen from Fig. 1, these concentrations of p-nitrophenol at this wave length transmit light according to the Lambert-Beer law.

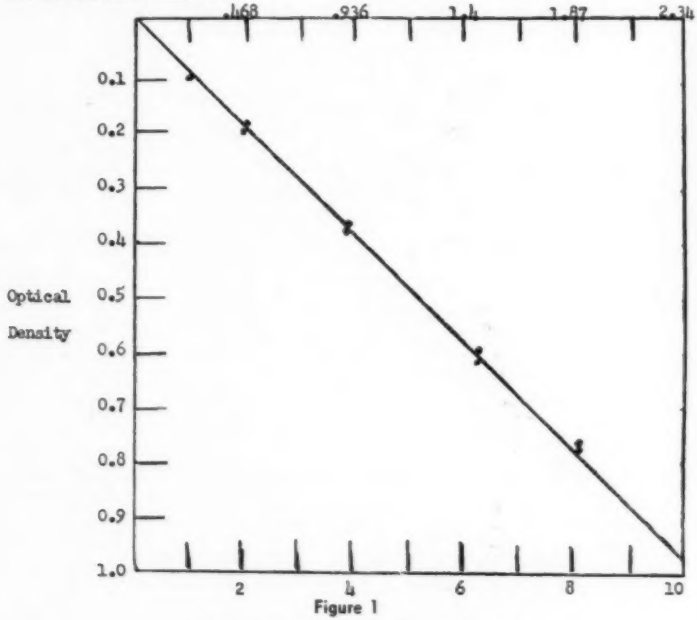

Figure 1

Calibration Curve for Serum Alkaline and Acid Phosphatase Activities

The millimol unit bears a peculiar relationship to the original Bodansky unit. The ratio of the latter unit to the millimol unit has been reported[10,11] to be approximately 1.8 in sera from children and adults for both alkaline and acid phophatases. An inspection of the data presented in the latter reference shows this to be generally true for values falling within the normal range. However, for elevated values the ratio is apparently reversed which necessitates a recalculation of the ratio values.

Analysis of their data shows that the ratios are actually less constant than reported, ranging from 0.49 to 1.93 instead of the stated range of 1.56 to 2.13.

The King Armstrong unit is reported to bear a ratio of approximately 7.3 to the millimol unit for alkaline phosphatase.[10] However, no data were presented to support this value. It will be seen in the following experiments that this ratio is too high and too variable to be of practical value when using the modified King Armstrong method[12] as a basis for comparison. No data regarding the ratio between these two units for acid phosphatase are available as far as this author has been able to ascertain.

Results

Serum phosphatases were run on an unselected group of hospital patients in order to compare the modified King Armstrong method and the p-nitrophenol method. It is evident from Table I (using the conversion factor 6.2) that there is fairly good agreement between the two methods for acid phosphatase (columns 2 vs. 4), but the ratio between these units as shown in the last column is not constant. Most of the values fall between 3 and 9 averaging 6.7 in our hands, as compared with the reported value of 6.2.

On the other hand, this ratio is of no value in comparing the activity of alkaline phosphatase as determined by the two methods. The average ratio of King Armstrong units to mM units is 2.49 ranging between 1.35 and 4.15.

The ratio 6.2 is based on normal values ranging between 3.7 and 13.1 units for alkaline phosphatase as reported originally by King and Armstrong.[9] Using the modified King Armstrong method[12] we have found these values to range between 3.0 and 8.0 units. Several other laboratories in this area using this modified procedure have also obtained much lower values (personal communications).

Alkaline Phosphatase—Normal Range
Modified King Armstrong Method

Laboratory A	2.0 - 4.0
Laboratory B	1.5 - 4.0
Laboratory C	2.5 - 9.0
Laboratory D	1.0 - 5.0

Two laboratories reported that they used the Bodansky method for alkaline phosphatase because the modified King Armstrong method was unsatisfactory in their hands.

Thus the ratio 6.2 is valid for alkaline phosphatase only when the normal range is considered to be between 3 and 13 units, which we have found not to be true when the modified King Armstrong method is used.

TABLE I

Comparative Results with King Armstrong and p-Nitrophenyl Phosphate Procedures

Serum No.	K.A. Units	mM Units	mM Units x 6.2	Ratio K.A. Units to mM Units
ACID PHOSPHATASE				
1	5.9	0.93	5.8	6.34
2	2.2	0.56	3.5	3.93
3	5.9	0.74	4.6	7.97
4	3.4	0.66	4.1	5.15
5	2.6	0.29	1.8	6.20
6	4.4	0.53	3.3	8.31
7	5.9	0.71	4.4	8.31
8	0.7	0.09	0.5	7.78
9	1.6	0.51	3.2	3.17
10	1.4	0.40	2.5	3.50
11	2.6	0.29	1.8	8.96
12	0.7	0.09	0.5	7.78
13	1.4	0.14	0.9	9.93
Average	....	....	....	6.72
ALKALINE PHOSPHATASE				
1	5.4	2.4	14.8	2.25
2	5.4	1.3	8.1	4.15
3	4.2	1.8	11.1	2.33
4	9.6	5.5	34.1	1.75
5	22.5	9.5	58.9	2.37
6	6.3	3.4	21.0	1.88
7	3.7	2.0	12.4	1.35
8	5.0	3.6	22.3	1.40
9	7.3	3.7	22.9	1.98
10	7.8	3.0	18.6	2.60
11	8.7	2.9	18.0	3.00
12	16.3	4.9	30.4	3.34
13	6.0	2.0	12.4	3.00
14	3.8	2.2	13.6	1.73
15	3.0	1.8	11.2	1.67
16	3.7	2.2	13.6	1.68
17	3.3	1.6	9.9	2.06
18	4.2	1.7	10.5	2.47
19	7.3	2.3	14.3	3.17
20	5.9	2.4	14.9	2.46
21	5.9	1.9	11.8	3.10
22	5.3	1.8	11.2	2.94
23	4.6	1.3	8.1	3.54
24	3.9	1.1	6.8	3.54
Average	....	....	....	2.49

Accuracy of the Method

Duplicate analyses agree on the average within 5 per cent for those values lying within the normal range. A small series of duplicate analyses by two different laboratories* agreed on the average within 8 per cent.

Normal Values

In a series of 51 normal male medical students acid phosphatase activity values ranged between 0.04 and 0.70 with an average of 0.35 mM units. In a series of 75 men, Andersch[11] found a range from 0.03 to 0.63 with an average of 0.27 mM units.

* We are grateful to the Barnes Hospital Laboratory for running duplicate samples for us.

In the same group of 51 students, alkaline phosphatase activity values ranged from 0.8 to 2.9 with an average of 1.6 mM units. Normal adult serum has previously been reported to range from 0.8 to 2.3 mM units.*

Discussion

Bessey, Lowry, and Brock,[10] with reference to alkaline phosphatase, make the following statement: ". . . in a personal communication, Dr. S. H. Jackson, Children's Hospital, Toronto, reported that the ratio of King-Armstrong units (mg. of phenol split from phenyl phosphate per 100 ml. of serum per hour) to mM units, is 7.3, with a variance of 8 per cent. With rat sera we have found in agreement with Fujita[13] that a much lower ratio is obtained. This observation deserves further investigation." From our own data referred to above it would appear that there is an average acid phosphatase activity ratio of approximately 6.7, and an alkaline phosphatase activity ratio of approximtely 2.5.

It has been called to our attention that many laboratories, in addition to our own, experienced wide discrepancies between the modified King Armstrong method and the p-nitrophenol method when using the factor 6.2 to convert mM units of alkaline phosphatase to King Armstrong units. We believe that it is erroneous to use conversion factors since such factors are based on an average of ratios which are too widely spread to give accurate results. Each method utilizes a different substrate with its own characteristic optima of pH, temperature, and time of hydrolysis. Hence, normal values must be established for each substrate.

Although it may be cumbersome, we feel that the designation of a new term will differentiate this method from its counterparts. The term "Bessey-Lowry unit" is suggested since this method is essentially the same as that developed by Bessey, et al., in 1946.[10]

The speed and simplicity of the method tend to enhance its value as a clinical laboratory procedure. The elimination of the precipitation and filtration steps common to other methods, and the elimination of time-consuming periods of waiting for color development will immediately appeal to the busy medical technologist. Mechanical errors are reduced to a minimum which tends to supplement the inherent accurary of the method.

Summary

Procedures are outlined for the estimation of serum alkaline and acid phosphatases using p-nitrophenyl phosphate as the substrate. The accuracy, speed, and simplicity of the methods highly recommend them for use in the clinical laboratory.

Normal values for the acid phosphatase activity of the serum

* Bulletin 104, Sigma Chemical Co.

in 51 normal men range from 0.04 to 0.70 mM units and for alkaline phosphatase activity from 0.8 to 2.9 mM units. A factor of 6.2 may be used to convert mM units of acid phosphatase to King Armstrong units, but the reported ratio of 6.2 does not apply to alkaline phosphatase because the normal values by the modified King Armstrong method are much lower than those originally reported by King and Armstrong. The factor of 2.5 may be used to convert mM units of alkaline phosphatase to King Armstrong units.

It is recommended that conversion factors be eliminated and that the term "Bessey-Lowry unit" be used to express phosphatase activity by the p-nitrophenol method.

Acknowledgment

The author wishes to thank Dr. R. O. Muether, Associate Professor of Internal Medicine and Director of Laboratories, Firmin Desloge Hospital, St. Louis University School of Medicine, and Miss Patricia Ann Thomas, B.S., MT (ASCP), Firmin Desloge Hospital Laboratory, for their assistance in securing blood specimens; and Dr. R. O. Muether and Dr. Philip Katzman, Professor of Biochemistry, St. Louis University School of Medicine, for valuable suggestions rendered in the preparation of the manuscript.

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It has recently been called to our attention that in the preparation of the calibration curve using p-nitrophenol as the standard, many individuals have been unable to obtain a straight line relationship between the optical density and the mM units of phosphatase activity. Subsequent standard curves prepared by us seem to bear out this finding. This need create no problem since one merely draws a curved line through the points obtained.

We would like to emphasize that when an acid phosphatase greater than 2.34 mM units or an alkaline phosphatase greater than 10 mM units is obtained one should repeat the test using half the amount of serum. The result is then multiplied by 2 to compensate for the smaller amount of serum used.

THE PATIENT

The patient is not an interruption of our work—he is our work.

The patient does us a favor when he calls; we are not doing him a favor by serving him.

The patient is dependent upon us; our reputation is dependent upon him.

The patient is not some one to argue with— but someone to comfort.

The patient is not a cold statistic; he is a flesh and blood human being with feelings and emotions like our own.

The patient is a person who brings us his illness. It is our duty to justify his faith in us.

The patient is part of our business—not an outsider.

The patient is the most important person in the hospital.

REMEMBER ALWAYS . . . *If people did not become ill there would be no need for our profession.*

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EVALUATION OF "DROP" TECHNIQUES FOR THE DETECTION OF HEMAGGLUTININS AND HEMOLYSINS IN TUBERCULOUS SERA

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The examination of tuberculous sera by the hemagglutination of tuberculin-sensitized erythrocytes, as reported by Middlebrook and Dubos,[1] and the hemolytic modification of this test, described by Middlebrook,[2] has been the subject of numerous investigations.

Of the various procedures reported, the hemagglutination technique of Scott and Smith[3] has been widely used, principally because of the standard antigen suggested by these investigators. A slide modification of this technique has been reported,[4] but was not found to be practical for some studies by the authors. Fisher[5] and Levine[6] have described alterations in procedures which either conserve reagents or shorten the time required for completion of the tests.

The success achieved employing drop techniques with various other hemagglutinating systems suggested an application to the testing of tuberculous sera with O.T.-sensitized cells. The purpose of this paper is to present a comparison between the results obtained using the "drop" modifications and the usual serologic procedures, with 54 sera.

Materials and Methods

The reagents were prepared in essentially the same manner as described by the above authors.

(1) *Sheep Cells*—Sheep blood was drawn into Alsever's solution and the cells separated by centrifugation at 2000 rpm for 10 minutes. The cells were recovered and washed three times with 6 to 10 volumes of phosphate-buffered saline solution (pH 7.4) and stored at 4° C as "packed" cells.

(2) *Sensitization of Cells with Old Tuberculin*—To 0.1 ml of packed cells was added 5.6 ml of buffered saline and 0.4 ml of Old Tuberculin (Lederle)* which had been concentrated to four times the International Standard. This resulted in a 1:15 dilution of O.T. The mixture was incubated for 2 hours in a 37° C water bath, with frequent agitation. The cells were removed from suspension by low-speed centrifugation (1500 rpm for 10

[1] The Department of Bacteriology of The Ohio State University.
[2] Battelle Memorial Institute.
* Provided through the courtesy of Dr. H. D. Piersma, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

minutes), washed three times with 6 to 10 volumes of buffered saline solution, and resuspended with saline to a 0.5% concentration. These sensitized cells were stored at 4° C for as long as three or four days.

(3) *Absorption of Sera*—The sera to be tested were diluted with equal quantities of buffered saline, inactivated at 56° C for 30 minutes and cooled to room temperature. One-tenth ml of washed and packed, normal sheep erythrocytes was added for each 1.0 ml of diluted serum. The cell-serum mixture was placed in a 37° C water bath for 30 minutes. After centrifugation at 1500 rpm for 10 minutes, the supernatant fluid was removed and re-absorbed by the same procedure. The absorbed sera were stored at —20° C.

(4) *Absorption of Complement*—Dehydrated pooled guinea pig serum was reconstituted with the diluent provided by the manufacturer.* A single absorption of complement was carried out by adding packed sheep cells in a 0.1 volume concentration and incubating the cell-complement suspension at 4° C for 20 minutes. After centrifugation, the supernatant fluid was diluted 1:3 with buffered saline. The absorbed, diluted complement was always used the same day.

(5) *Hemagglutination and Hemolytic Tests*—("Standard" tests) To each in a series of tubes (12 x 75 mm) was added 0.4 ml of a twofold saline dilution of the test serum (1:2 through 1:4096) and 0.4 ml of the 0.5% sheep cells, previously sensitized with O.T. as described above. The tubes were incubated at 37° C for 2 hours, after which a preliminary reading for the titration end point was made by determining the last serum dilution in which the sedimented cells would not disperse readily with agitation. All tubes were vigorously shaken and reincubated at room temperature overnight, followed by a final reading. Serum and cell controls were included.

The hemolytic test was performed in the same manner as the hemagglutination test except that 0.2 ml of the absorbed, diluted complement was added to the cell-serum mixture and the test was terminated after incubation for 2 hours at 37° C. An additional control for complement was included. The last dilution exhibiting complete lysis served as the end point of the titration.

The same concentrations and relative proportions of reagents were employed in the "drop" modifications. Two drops of saline were introduced into each tube in a series, except the first, by means of a capillary pipette or medicine dropper. Two drops of the serum dilution (already diluted 1:2) were placed in the first

* Sold as dehydrated complement by the Texas Biological Laboratories, P. O. Box 722, Fort Worth, Texas.

TABLE I
Hemagglutination and Hemolytic Values of 54 Sera Tested by "Standard" and Drop Techniques

Source of Antisera: Rabbits injected with	Hemagglutination Titer*		Hemolytic Titer*	
	"Standard"	Drop Technique	"Standard"	Drop Technique
M. tuberculosis var. *bovis* (Ravenel)	128	256	1024	1024
	16	32	128	128
	128	64	4096	2048
	128	64	2048	1024
	256	256	1024	1024
	8	16	32	64
	32	32	512	512
	2	8	64	128
	64	64	1024	512
	16	32	256	128
	16	32	64	128
	16	32	256	256
	16	16	256	256
	16	32	1024	1024
	64	128	256	256
	8	8	32	64
	8	4	32	64
	16	16	128	128
	16	16	64	128
	2	—	4	8
	2	2	4	8
	—	—	2	4
	16	8	32	32
	8	8	16	16
	16	8	64	32
	16	16	128	128
	8	4	8	4
	4	2	8	4
BCG	2	—	2	—
	128	128	512	1024
	2	4	4	8
	32	2	128	32
	—	—	4	—
	2	2	—	—
P.P.D.	16	8	32	32
	2	—	—	—
	2	—	4	4
	16	8	64	32
	16	16	64	64
	2	2	8	2
	2	—	8	4
	8	4	32	16
	8	4	32	16
	16	16	128	128
	8	2	32	8
	2	4	4	16
M. phlei	1024	512	2048	4096
	512	256	4096	4096
Control	—	—	—	—
	—	—	—	—
	—	—	—	—
	—	—	—	—
	—	—	—	—
	—	—	—	—
	—	—	—	—

* Titers expressed as reciprocal of the dilution recorded as the end point.

and second tubes. The saline-serum dilution in the second tube was mixed by shaking the tube, and two drops of the dilution were transferred to the third tube, and so on. To these doubling dilutions of serum were added two drops of the sensitized cell suspension. If both the hemagglutination and the hemolytic tests were to be performed, the serum dilutions were either prepared

in duplicate or a single set of dilutions was made with twice the quantity of reagents (four drops of each) and two drops of each dilution was transferred into the corresponding tube in a second series. One drop of absorbed and diluted complement was added to each serum dilution for the hemolytic test plus the complement control. All tubes were shaken and incubated for 30 minutes in a 37° C water bath. The end point of the lytic test was the last dilution of serum in which definite hemolysis was observed. The end point for the hemagglutination titrations was first determined by observation of the sedimented cell pattern. Tubes bracketing this end point were then centrifuged at 1500 rpm for 1 minute followed by a final reading based on definite aggregation. The centrifugation was found to be necessary since false end points were frequently found if pattern was the sole criterion.

Results

Fifty-four rabbit sera were tested for their hemolytic and hemagglutinating properties. For purposes of comparison, the techniques employing larger quantities of reagents were chosen as the "standard" procedures and the results obtained by the drop techniques were evaluated accordingly.

The sera were collected from normal rabbits, animals which had received infectious doses of *Mycobacterium tuberculosis* var. *bovis* (Ravenel) or had been injected with P.P.D., *M. phlei* or BCG. Differences in titers within a series have no significance, since the sera were not procured at similar intervals following treatment.

The results of this study are recorded in Table 1 where it may be seen that with but few exceptions, the values obtained by the drop techniques agreed to within one dilution with those found with the "standard" procedures.

Of the hemagglutination titers recorded for the drop technique, agreement to within one dilution occurred in 94.3% of the titrations and to within two dilutions in 98.1% of the tests. The hemolytic titers agreed to within one dilution in 90.7% of the tests and to within two dilutions in all tests. In no instance did a normal serum show evidences of hemolytic or hemagglutinating activity. As will be noted, there were no false positive tests obtained with the "drop" techniques, and the variations between results observed by the two procedures with a single serum are no greater than commonly found with duplicate tests.

Comments

Serologic investigation of tuberculous sera often requires volumes of serum which might be difficult or inadvisable to obtain. The technique described in this paper obviously re-

sults in conservation of sera without sacrificing specificity or sensitivity. In spite of the small quantities of reagents, titration end points can be readily determined, and in the hemagglutination technique, more precisely than with the procedure employed as the "standard."

In addition, both the hemolytic and the hemagglutination tests are completed in 30 minutes, so that uniformity of incubation is accomplished.

Summary

(1) "Drop" modifications of the hemagglutination and hemolytic tests for tuberculous sera have been described.

(2) The results obtained with the modifications correlate well with the more conventional procedures, without alteration of specificity and sensitivity.

(3) These modifications have advantages insofar as very small quantities of sera and other reagents are required, and the time of testing is limited to 30 minutes.

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FUNDAMENTALS FOR A STATE PUBLICATION

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The last few years have witnessed a change in the type of state publications issued by medical technologists. The mimeographed single sheet was first in evidence, then several such were stapled together; folders appeared here and there. At this writing an ever increasing number of States are publishing small-sized journals. From an original lay-out of news items and announcements, there has developed a fairly well organized and professionally printed medium, which contains, in addition to the above, articles on technique, professional and public relations, recruitment, and various other aspects of medical technology.

The booklets are edited by registered medical technologists. At stated intervals they are sent, not only to the members of a particular state society, but also to various institutions and individuals. The last-mentioned are non-members who are, or should be, interested in a profession which plays such an active and responsible part in the service of medicine. The success of the journal lies in the efforts and in the interest of the members of the society publishing it.

The American Journal of Medical Technology is edited, printed, and published by professional people for the members of a national organization. The state publication provides a medium within a given locale by which medical technologists strive not only to maintain and to improve professional performance among its members, but also endeavor to establish and increase interest for their growing profession. Just as medical technology serves others, it should also be recognized and evaluated by them. If medical technologists fail to make the public acquainted with their existence, if they do not make them aware of the services they render, then they cannot expect others to appreciate and to value the importance of their profession; further, they cannot expect to increase in numbers. No one can do this for them unless the medical technologist has first led the way.

With this viewpoint in mind, it is extremely necessary that a medical technologist appointed to be editor of the state publication be an individual vitally interested in the medical technology profession; he must be capable and willing to assume the responsibilities that the publishing of such a journal entails, and he must strive to improve each issue. Granted that such an editor is found, a staff must be appointed, the members of which are willing to give time and effort with the editor, to further the work of the profession. There is no personal gain attached except the inward satisfaction that one is giving to the profession what can be expected of a registered technologist, but what is not necessarily demanded. If the staff works consistently—conscientiously—the publication cannot help but serve the purpose for which it has been established.

One of the important phases of a state publication, which spells success or failure is the active participation and contribution that its members can make. Without these, the publication begins to lose character, and appears to be like a family photo album without pictures of the family. This is the only concrete way in which there can be a definite interchange of ideas, views, and techniques.

The staff's function, then, is to plan the various issues of the journal during the year. It may be found feasible to have a definite purpose set for each issue, and that issue designated such, *i.e.*, Hospital, Recruitment, Membership or Convention Issues. As topics are received, they are assigned to the volume for which they are particularly adapted. A message from a pathologist in each number enhances the reading value of the publication. Other guest writers such as hospital

administrators, prospective students, graduates, and doctors specializing in the various branches of medicine may be contacted for contributions to particular issues. Members of the society may participate, not only by writing, but by securing patrons and ads to finance the project, by preparing envelopes for distribution, etc. Taking an active part leads the individual technologist to share in the responsibilities of the society of which he is a member. The group can in this way improve the journal as well as help one another to develop personally and professionally.

In trying to form the make-up of a publication, it is wise to study what is written in other state journals; in time the editors may become interested in comparing notes and pooling experiences with fellow-technologists across the country, thereby promoting a unity that will definitely help the national Society.

After a ghost-sketch of the current issue is readied, it is a good idea to have each staff member submit his comments and suggestions. With the composite thinking of the group, erroneous statements are not likely to be printed. Attractive photographs and drawings are welcome as they afford more interesting presentation than all solid black and white prints.

Particular help may be gained from the printer in arranging the format. Directives from a printer suggest that all copy submitted be typed and checked to be sure it is correct. A close estimate of the amount of copy to fit a given space is sometimes hard to determine, but it is better to be a little short than to have too much copy. All copy should be identified and keyed (or marked) as to location in the booklet. Proofs should be read and corrections should be marked. Any illustrations or pictures should be keyed (marked) as to location. Any special pages should be layed out or diagrammed to show the effect desired. If any advertiser wants a specially designed ad, he should give the printer a layout.

Plenty of time should be allowed for the printing of any magazine. A book like the *Techni-Caller* (Ark.) takes about twenty days, but an allowance of thirty days is desirable.

The paper and cover is selected with quality as well as weight in mind. A shade of difference in weight means additional postage. Envelopes, even though increasing costs, make the journal a bit more inexpensive. Having them addressed and stamped when the journals arrive is a definite advantage.

The mailing list of a state publication devoted to interest the members of the society, as well as related groups, includes routinely all active and associate members, the pathologists throughout the state, the office and the officers of the National Society, editors of similar publications, and presidents of the states not having publications. Particular issues are sent to the hospitals, to schools, and to vocational and counseling agencies.

The value of a state publication should never be underestimated. Through such a medium, a strong link in a national chain which possesses strength to promulgate its basic mission can be provided.

Each of us must realize that our organization is made up of a limited number of registered medical technologists scattered throughout the entire state. It is sometimes difficult to maintain consistent district meetings—this in itself indicates that the strength must come from the entire state organization—each doing his or her part to enhance our present position. It is well to remember that the success of our journal depends on the efforts and interest of our entire state Society of Medical Technologists.

BOOK REVIEW

Methods of Biochemical Analysis, Volume 1
Edited by David Glick
Interscience Publishers, 1954. 509 pp. $8.50

This is the first of a series of annual review volumes which will be devoted to innovations and improvements in instrumentation, methods and techniques of biochemical analysis.

According to Dr. Glick's Preface, "The general plan followed in the organization of the individual chapters is a discussion of the background and previous work, a critical evaluation of the various approaches, and a presentation of the procedural details of the method or methods recommended by the author. The presentation of the experimental details is to be given in a manner that will furnish the laboratory worker with the complete information required to carry out the analyses."

Of particular interest to the medical technologist in this first volume is the chapter "The Assay of Urinary Neutral 17-Ketosteroids" by Lewis L. Engel, the chapter "Zone Electrophoresis" by Henry G. Kunkel which includes the technique of paper electrophoresis, and the chapter "Chemical Determination of Ascorbic, Dehydroascorbic, and Diketogluronic Acids" by Joseph H. Roe. The scope of the book extends into the clinical bacteriology laboratory for it contains a chapter on "Microbiological Assay of Antibiotics" by Roger C. Kersey and Frederick C. Fink.

The series should make a useful addition to the laboratory library.

—ESTER FREIER

THE GAVEL

In writing my initial message as president, it is appropriate first that I speak for all of you in giving a very special word of appreciation to the Florida technologists for an enjoyable, stimulating, and successful convention; in expressing our sincere gratitude for the capable leadership of the officers and the energetic work of the committees of the past year; and in thanking the many who, over the years by unselfish service, have guided us into an organization of creditable achievements and professional dignity.

Secondly may I take this opportunity to express some personal thoughts about membership in our organization. To me ASMT means a group of individuals; each one with ideas and ideals, each one with hopes and aspirations; yet all united by common interest in our profession. Each individual has accumulated experience—unique to himself and impossible to find elsewhere—which represents a potential of inestimable value for building for the future.

Furthermore the most important thing about any person is his attitude toward other people. In this regard it is well to remember that expression of thoughts and ideas is dependent on communication. Communication is difficult. Misunderstandings can lead to critical attitudes. Critical attitudes can lead to competition. Competition can lead to isolation. Isolation can lead to distrust. Distrust can lead to disunity.

How then can the potential of the individual experience, intelligence, energy, and ideals be utilized? The answer is in your hands. Express your thoughts and ideas. Be tolerant of others. Participate in professional activities. Use imagination and initiative. Cooperate with other professional groups. Continue to learn. Be proud of your profession. Give of yourself. Believe in yourself.

By sharing, learning, and working together, our purposes will become alive and meaningful. ". . . To promote higher standards in clinical laboratory methods and research; to elevate the status of those specializing in medical laboratory technique; to create mutual understanding and co-

operation between the medical technologists and physicians and all others who are employed in the interest of individual or public health; to promote the mutual aid and benefits of its members . . ."

—RUTH HOVDE

EDITORIAL

OUR FILM IS GOOD

A preview of the new recruitment film "Career: Medical Technologist" before a distinguished panel of medical technologists, pathologists and medical educators during the American Medical Association convention in San Francisco the end of June, brought nothing but kudos and acclaim of its excellence as a portrayal of the medical technologist and his chosen profession.

The film tells the story of Joan—a personable young high school girl who decides to be a medical technologist, and presents forcibly and artistically the reasons for her decision. It takes her through the various high points of the medical technologist's work, dramatically relating each one to the human problem that is being solved. The story ends as she completes her first emergency assignment alone.

The film is not finished as yet. The preview showing was at the "interlock" stage, where the film track and the sound track have not yet been permanently joined. This viewing by a group of experts for clearance was to discover changes that might have to be made at a stage where it could be done easily and inexpensively.

The experts agreed that no changes were needed so final prints will be ready for distribution immediately after the premiere of the film. This is scheduled for the opening reception of the International Congress of Clinical Pathology in Washington, D. C., September 6. Prints of this 24-minute color film that cost $30,000 to produce, will sell for $135 each.

In order to facilitate immediate shipment of prints to purchasers, the National Committee for Careers in Medical Technology is using its revolving fund for the quantity purchase of 100 prints for redistribution to State Societies of Medical Technologists, clinical pathologists, and to Approved Schools of Medical Technology. The price of $135.00 which was achieved through quantity ordering is considerably less than the $200 usually charged for 24-minute color movies.

It is quite likely that most state, and many local or district societies of medical technologists will want to buy a print—you will want to show the movie before high school and college audiences over the country. Ask for a copy on approval. Write to the National Committee on Careers in Medical Technology, 1785 Massachusetts Ave., N.W., Washington, D. C.

Urology Award—The American Urological Association offers an annual award of $1000 (first prize of $500, second prize $300 and third prize $200) for essays on the result of some clinical or laboratory research in Urology. Competition shall be limited to urologists who have been graduated not more than ten years, and to men in training to become urologists.

The first prize essay will appear on the program of the forthcoming meeting of the American Urological Association, to be held at the Biltmore Hotel, Los Angeles, California, May 16-19, 1955.

For full particulars write the Executive Secretary, William P. Didusch, 1120 North Charles Street, Baltimore, Maryland. Essays must be in his hands before January 1, 1955.

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